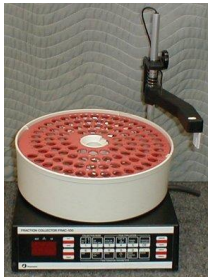
Beckman Coulter BioMek 3K
At BRDG Park R126B

Plate Washer, Bio-Plex Pro II
@BRDG used with
BioPlex Array reader

Liquid Handling:
BioMek 3000; Protocol
Plate Washer; SOP
Fraction Collector; Description



Fraction Collection
FRAC -100 @ FV

Select instrument picture to jump to that section of the SOP

Prepared by: Bob Morrison
STLCC- Instrumentation Specialist
Original June08, Latest Revision Aug2012

Beckman Coulter BioMek 3K
At BRDG Park R126B

Link to Beckman BioMek3000 User Manual – pdf (15MB)

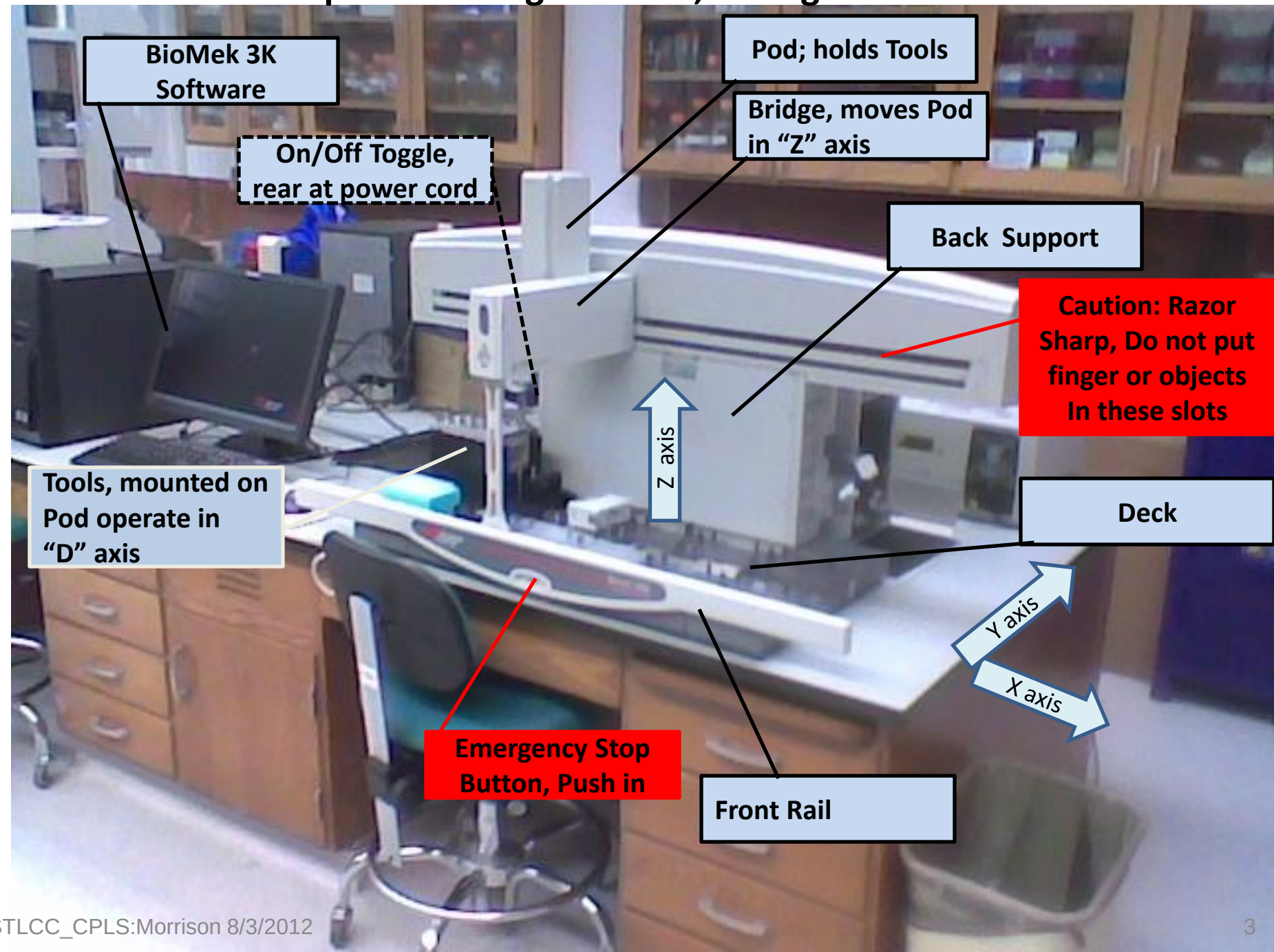
Link to Beckman BioMek3000 Quick Start Guide – pdf (2MB)

Link to Beckman BioMek3000 Software Manual – pdf (23MB)

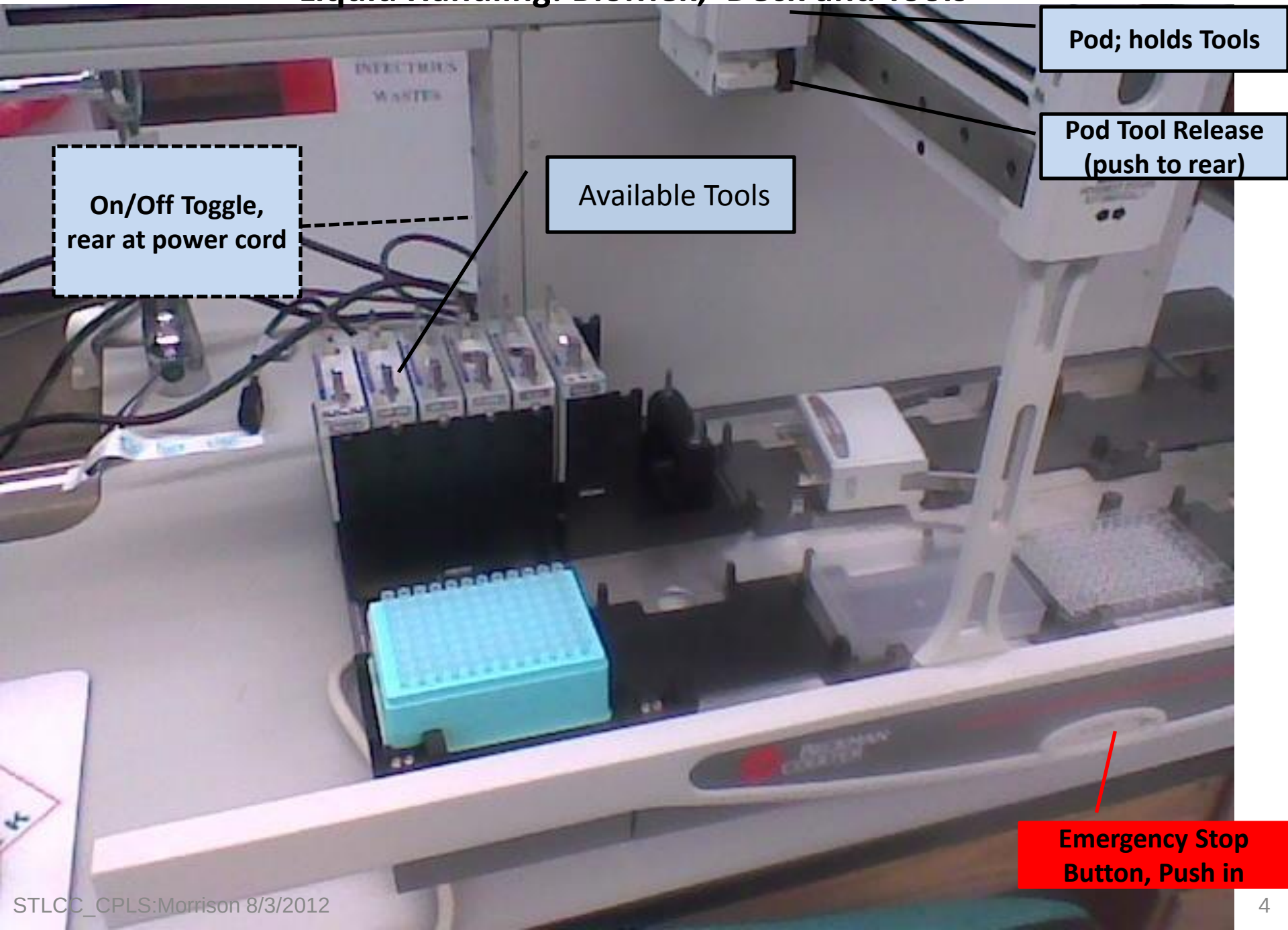
Host PC Login

Username: Biomek
Password: 15Biomek

Liquid Handling: BioMek, Configuration


BioMek 3K Software
Pod; holds Tools
Bridge, moves Pod in "Z" axis
On/Off Toggle, rear at power cord
Back Support
Caution: Razor Sharp, Do not put finger or objects In these slots
Z axis
Tools, mounted on Pod operate in "D" axis
Deck
Y axis
X axis
Emergency Stop Button, Push in
Front Rail

Liquid Handling: BioMek, Deck and Tools


Pod; holds Tools
Pod Tool Release (push to rear)
On/Off Toggle, rear at power cord
Available Tools
Emergency Stop Button, Push in

Liquid Handling: BioMek3000, Available Tools and Tips

- **P20 Single-Tip Pipette Tool** — a single probe tool for aspirating and dispensing 1-20 µL to a single well using P20 or P250 tips. Tested @ 1ul, %CV 1.2
- **P200L Single-Tip Pipette Tool** — a single probe tool for aspirating and dispensing 5-200 µL to a single well using P250 tips, with liquid-level sensing capabilities. Tested @ 5ul, %CV 4.1
- **MP20 Multi-Tip Pipette Tool** — an eight-probe tool for aspirating and dispensing 1-20 µL to eight wells simultaneously using P20 or P250 tips. Tested @ 1ul, % CV .6
- **MP200 Multi-Tip Pipette Tool** — an eight-probe tool for aspirating and dispensing 5-200 µL to eight wells simultaneously using P250 tips. Tested @ 5ul, %CV 1.1
- **WASH-1 200ul**, Tested @ 200ul, %CV 1.3
- **WASH-8 200ul**, Tested @ 200ul, %CV .6
- **Gripper**
- **Deck Framing/Calibration Tool**

Tips (Beckman) Currently On Hand for BioMek @ FV

- P250, AP96, 20ul, Barrier, Part# 717256, quantity 1
- P250, AP96, 125uL, Barrier, Part# 717253, quantity 1
- P250, Ap96, 240uL, Non-sterile, Part # 717251, quantity 4

Liquid Handling: Software , Screen Areas Defined

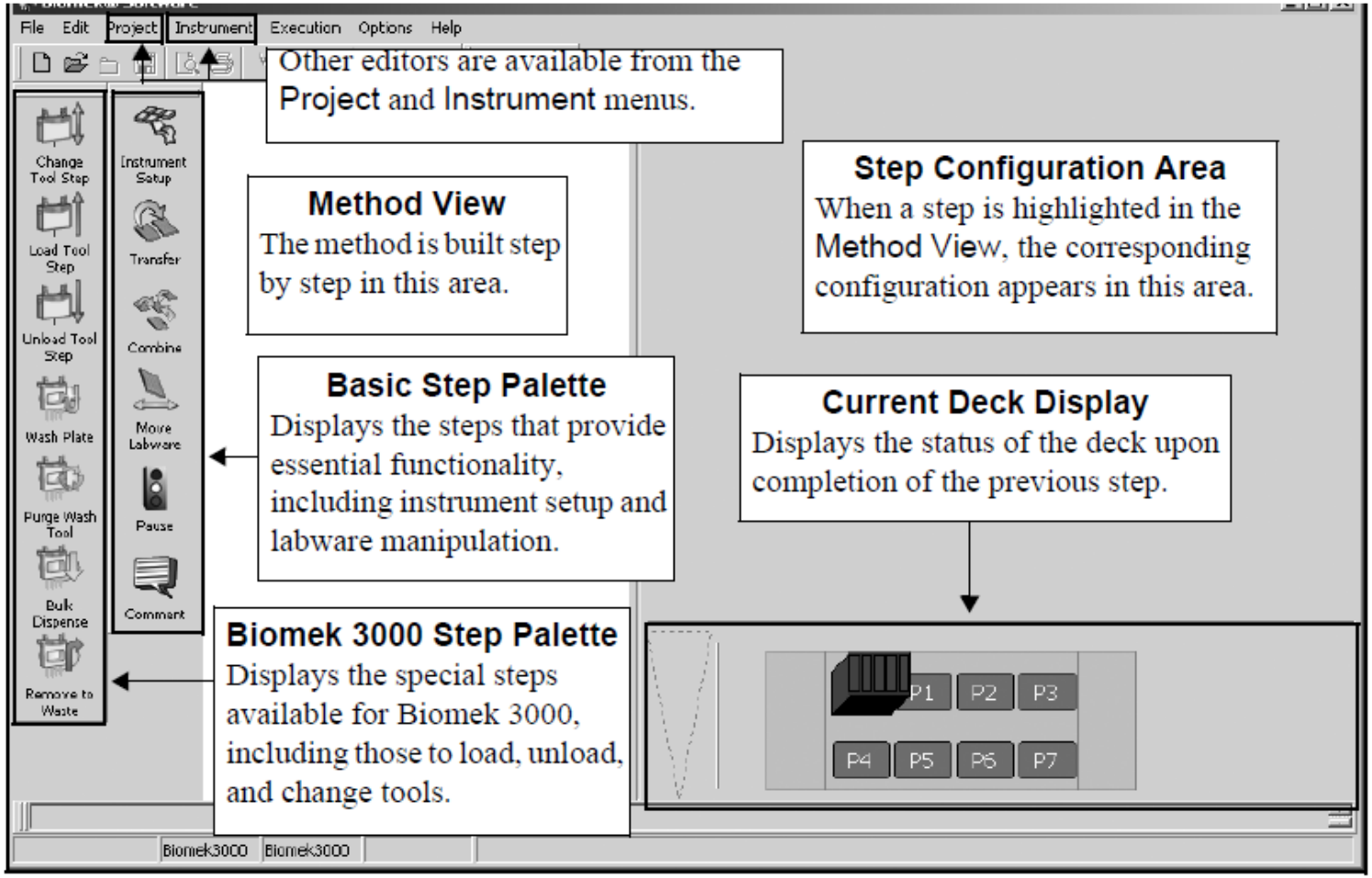
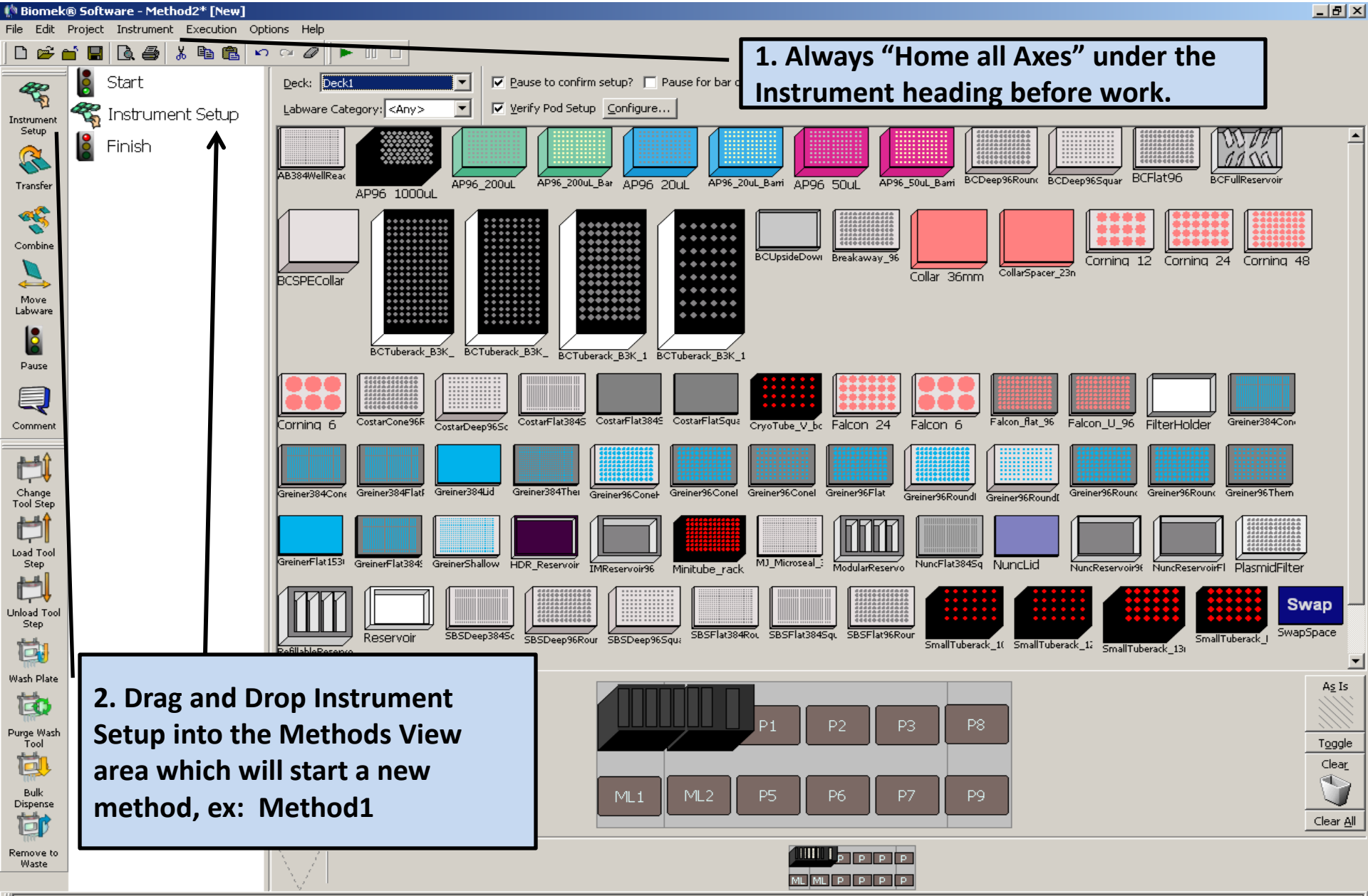
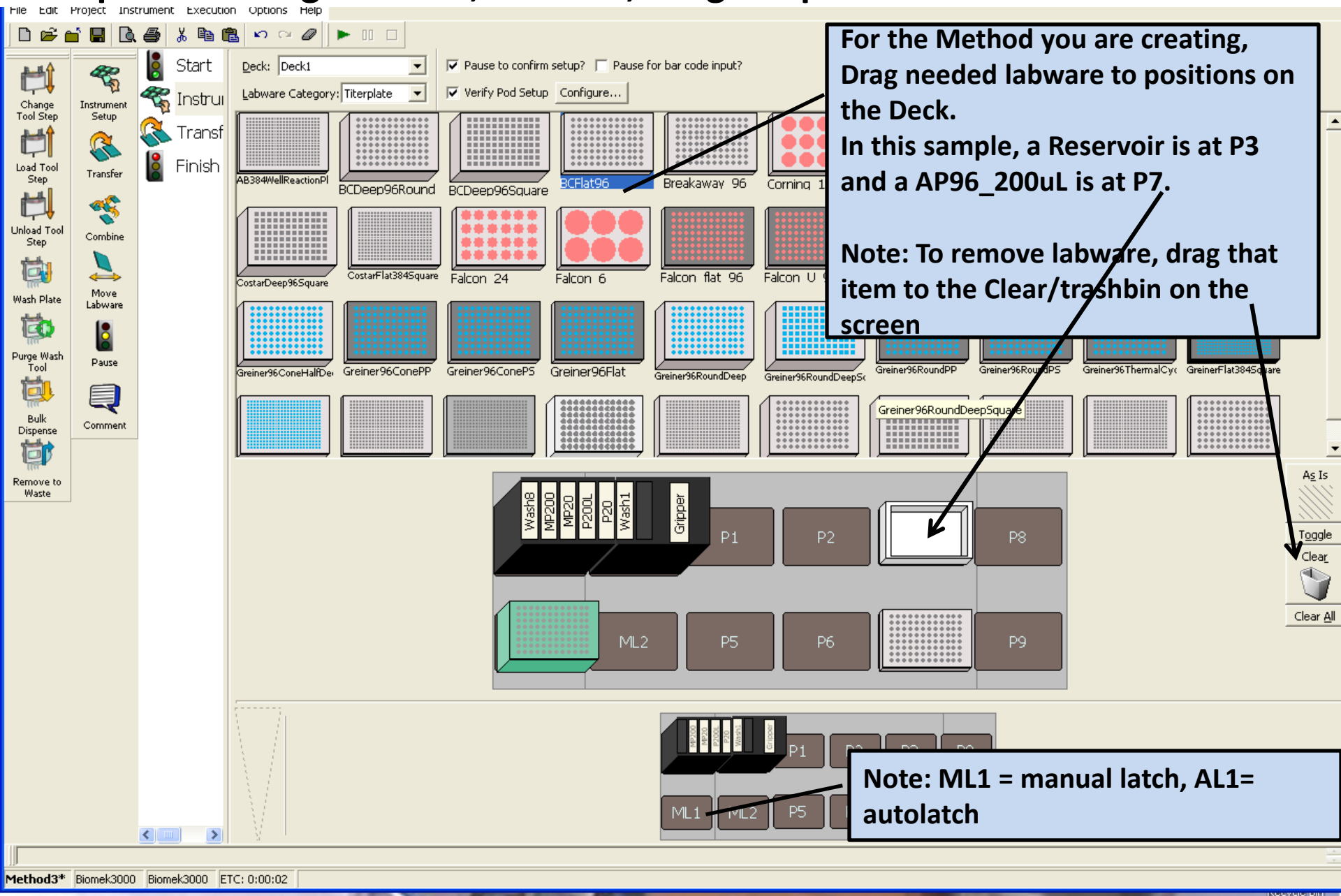

File Edit Project Instrument Execution Options Help
Other editors are available from the Project and Instrument menus.
Method View
The method is built step by step in this area.
Step Configuration Area
When a step is highlighted in the Method View, the corresponding configuration appears in this area.
Basic Step Palette
Displays the steps that provide essential functionality, including instrument setup and labware manipulation.
Current Deck Display
Displays the status of the deck upon completion of the previous step.
Biomek 3000 Step Palette
Displays the special steps available for Biomek 3000, including those to load, unload, and change tools.
Change Tool Step
Load Tool Step
Unload Tool Step
Wash Plate
Purge Wash Tool
Bulk Dispense
Remove to Waste
Instrument Setup
Transfer
Combine
Move Labware
Pause
Comment
P1
P2
P3
P4
P5
P6
P7
Biomek3000
Biomek3000

Figure 1-2. Biomek Software main editor

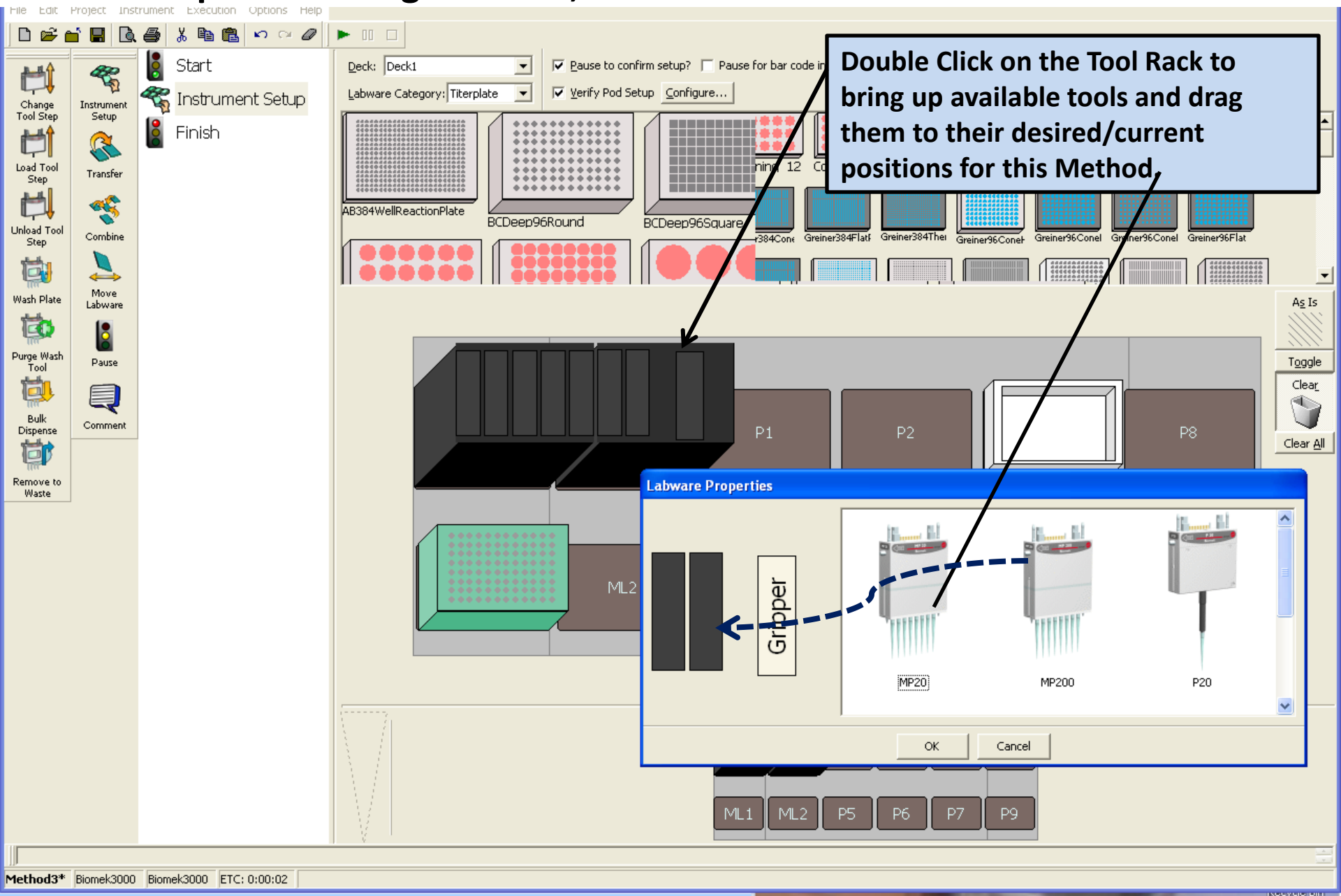
Liquid Handling: Biomek; Instrument Setup, Deck Labware Options



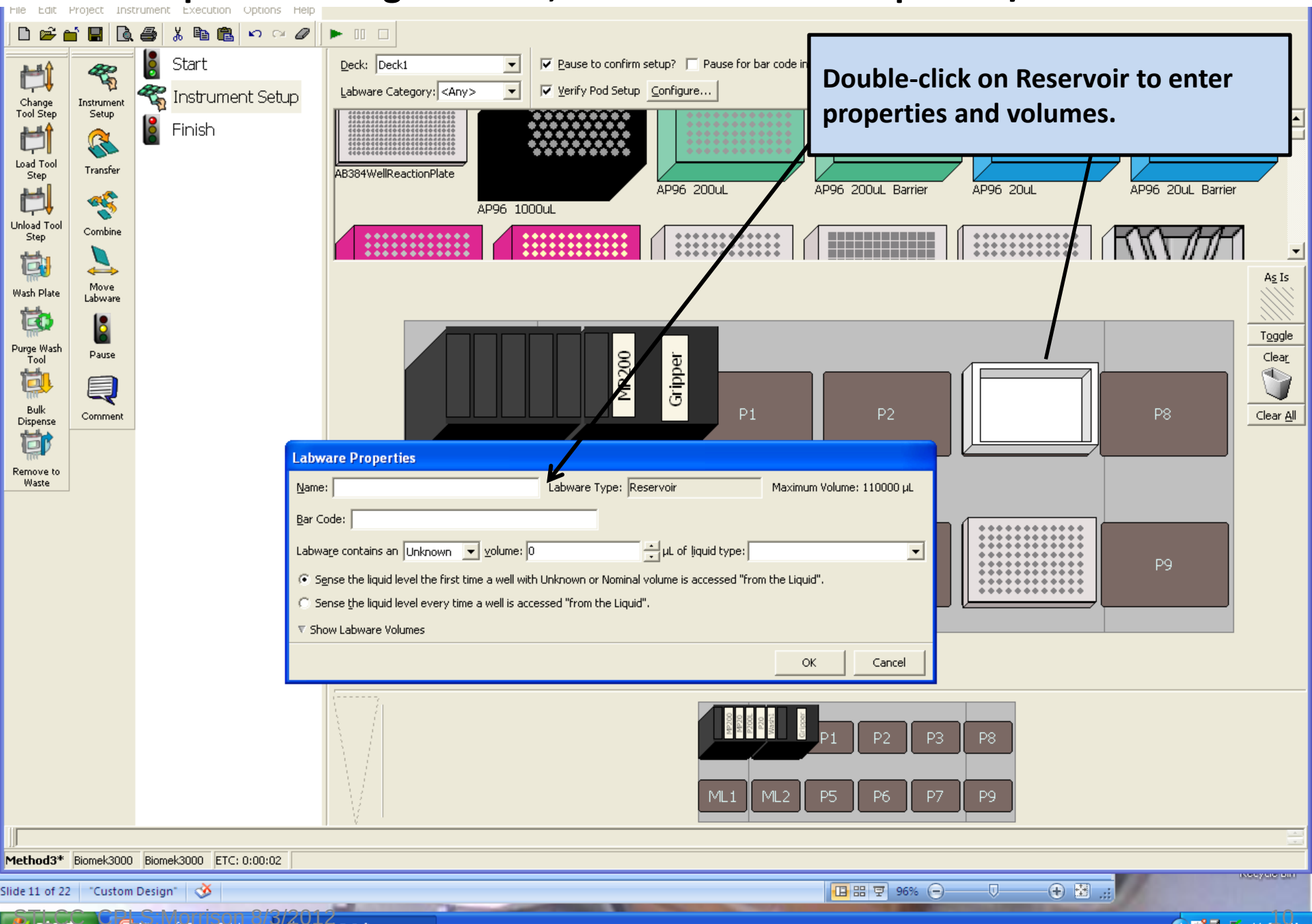
Liquid Handling: Biomek; Method, Drag-Drop Labware to Deck Locations



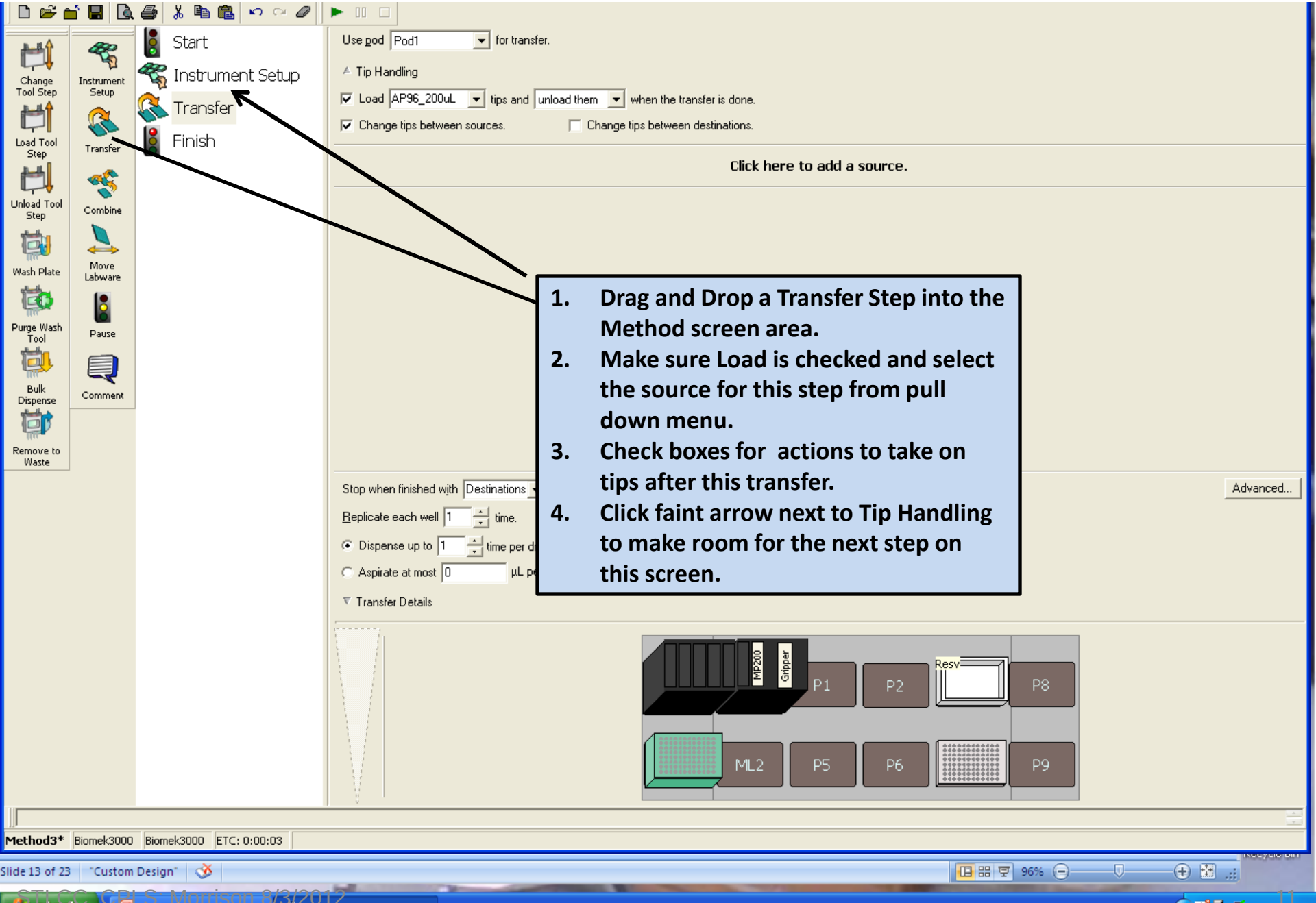
Liquid Handling: Biomek; Define Tools in their Rack Positions



Liquid Handling: Biomek; Define Labware Properties/volumes

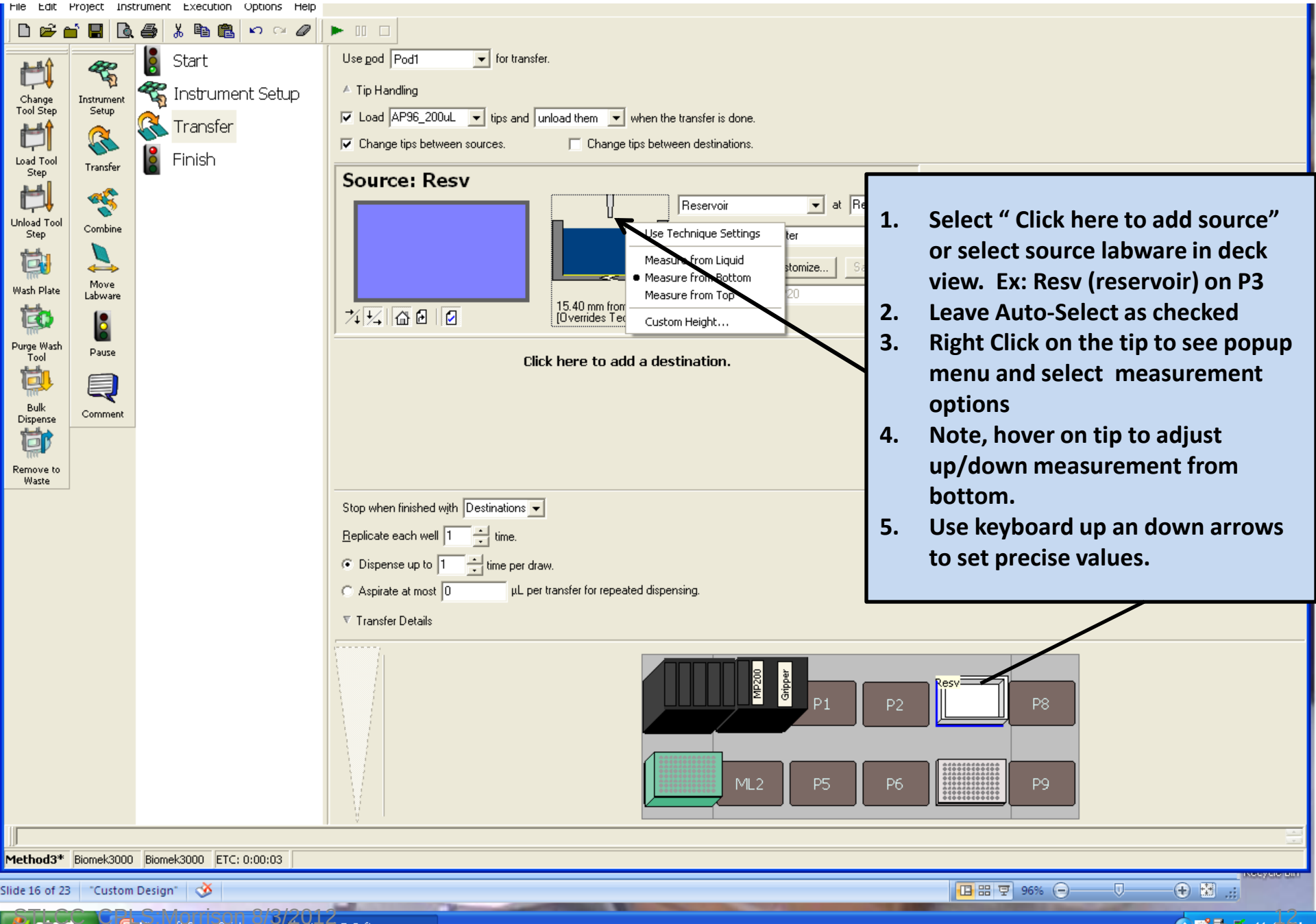


Liquid Handling: Biomek; Transfer Step, Define Tip Source



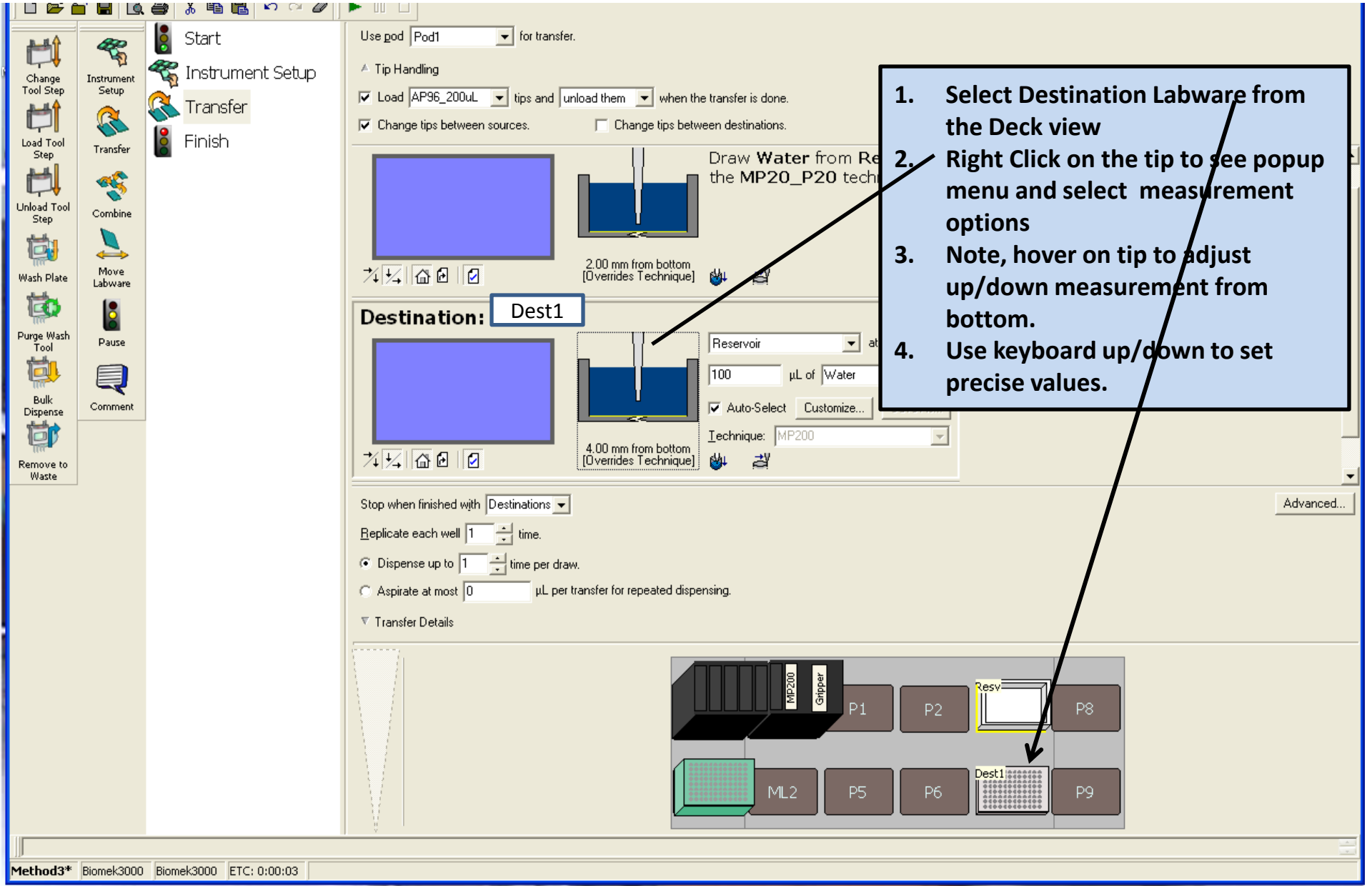
1. Drag and Drop a Transfer Step into the Method screen area.
2. Make sure Load is checked and select the source for this step from pull down menu.
3. Check boxes for actions to take on tips after this transfer.
4. Click faint arrow next to Tip Handling to make room for the next step on this screen.

Liquid Handling: Biomek; Define Labware Source



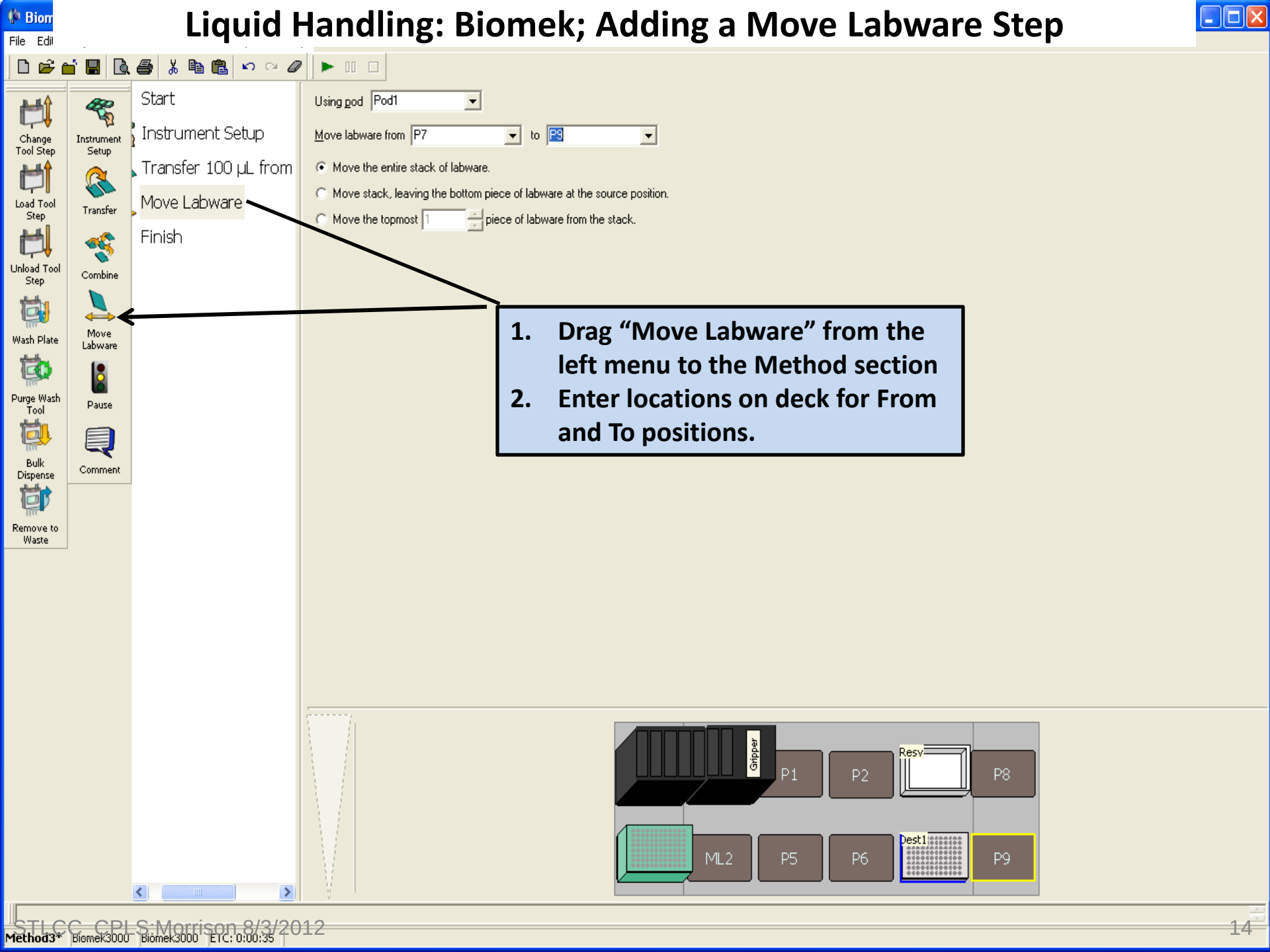
1. Select " Click here to add source" or select source labware in deck view. Ex: Resv (reservoir) on P3
2. Leave Auto-Select as checked
3. Right Click on the tip to see popup menu and select measurement options
4. Note, hover on tip to adjust up/down measurement from bottom.
5. Use keyboard up an down arrows to set precise values.

Liquid Handling: Biomek; Define Destination Labware

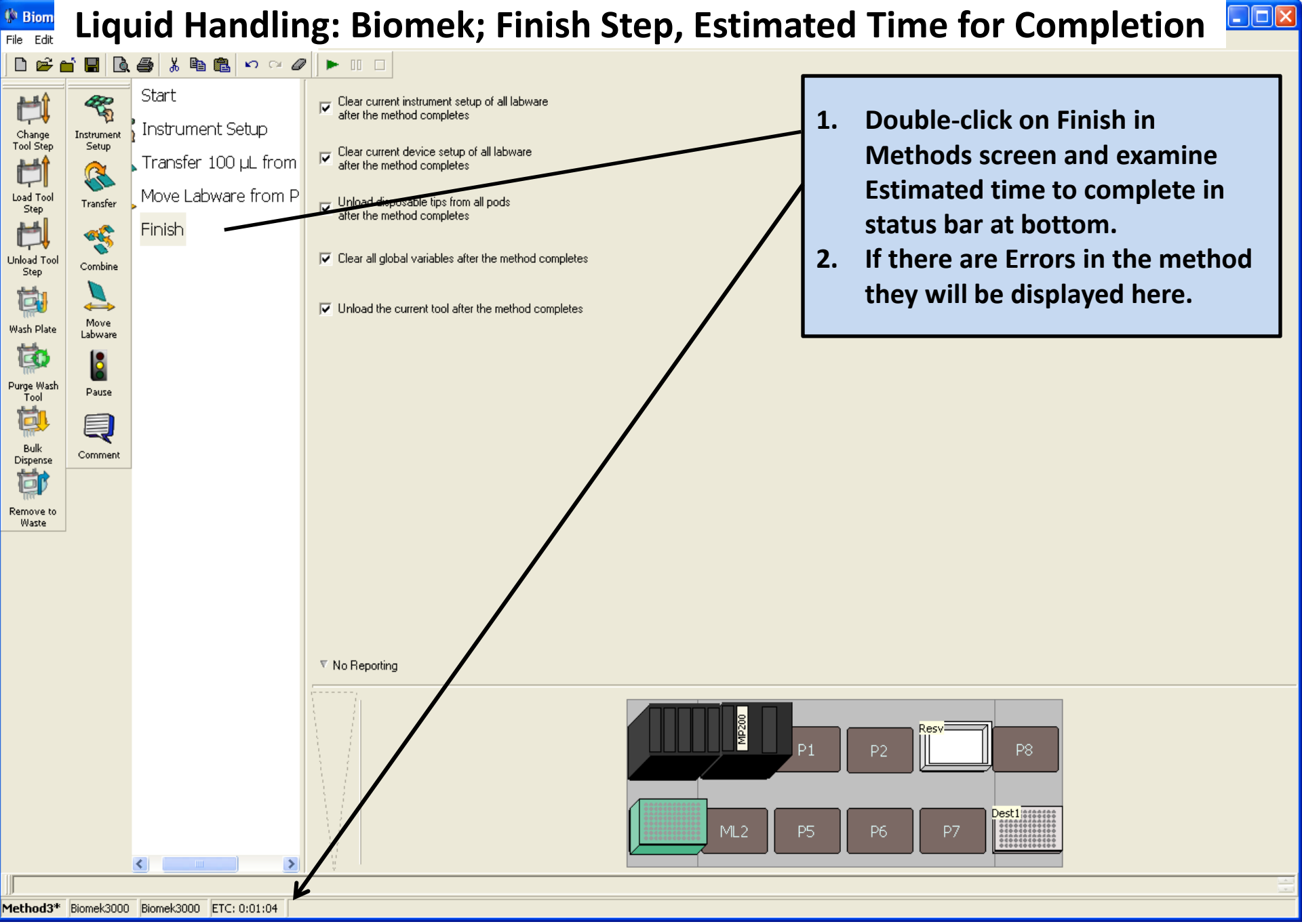


1. Select Destination Labware from the Deck view
2. Right Click on the tip to see popup menu and select measurement options
3. Note, hover on tip to adjust up/down measurement from bottom.
4. Use keyboard up/down to set precise values.

Liquid Handling: Biomek; Adding a Move Labware Step

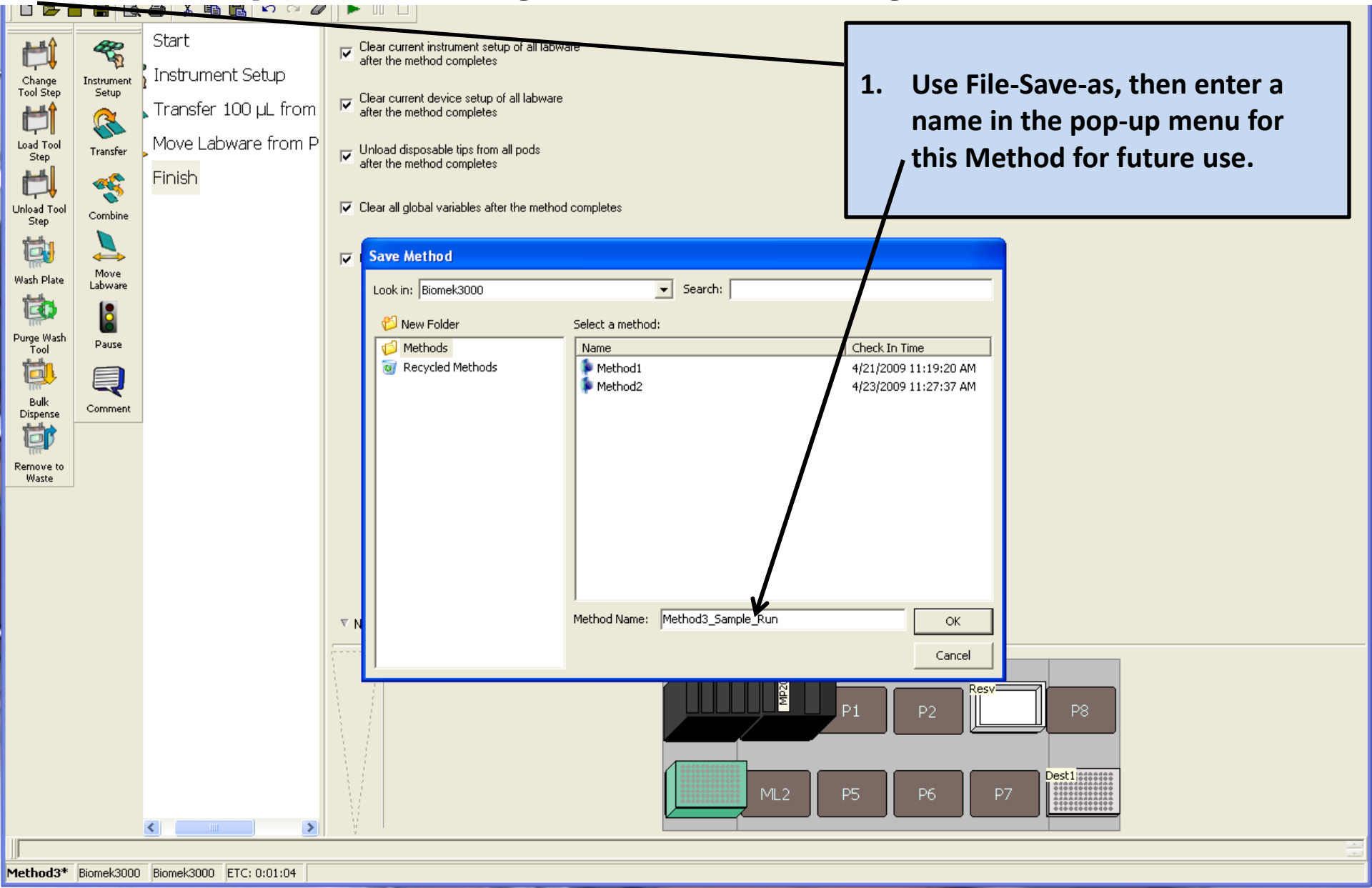


Liquid Handling: Biomek; Finish Step, Estimated Time for Completion

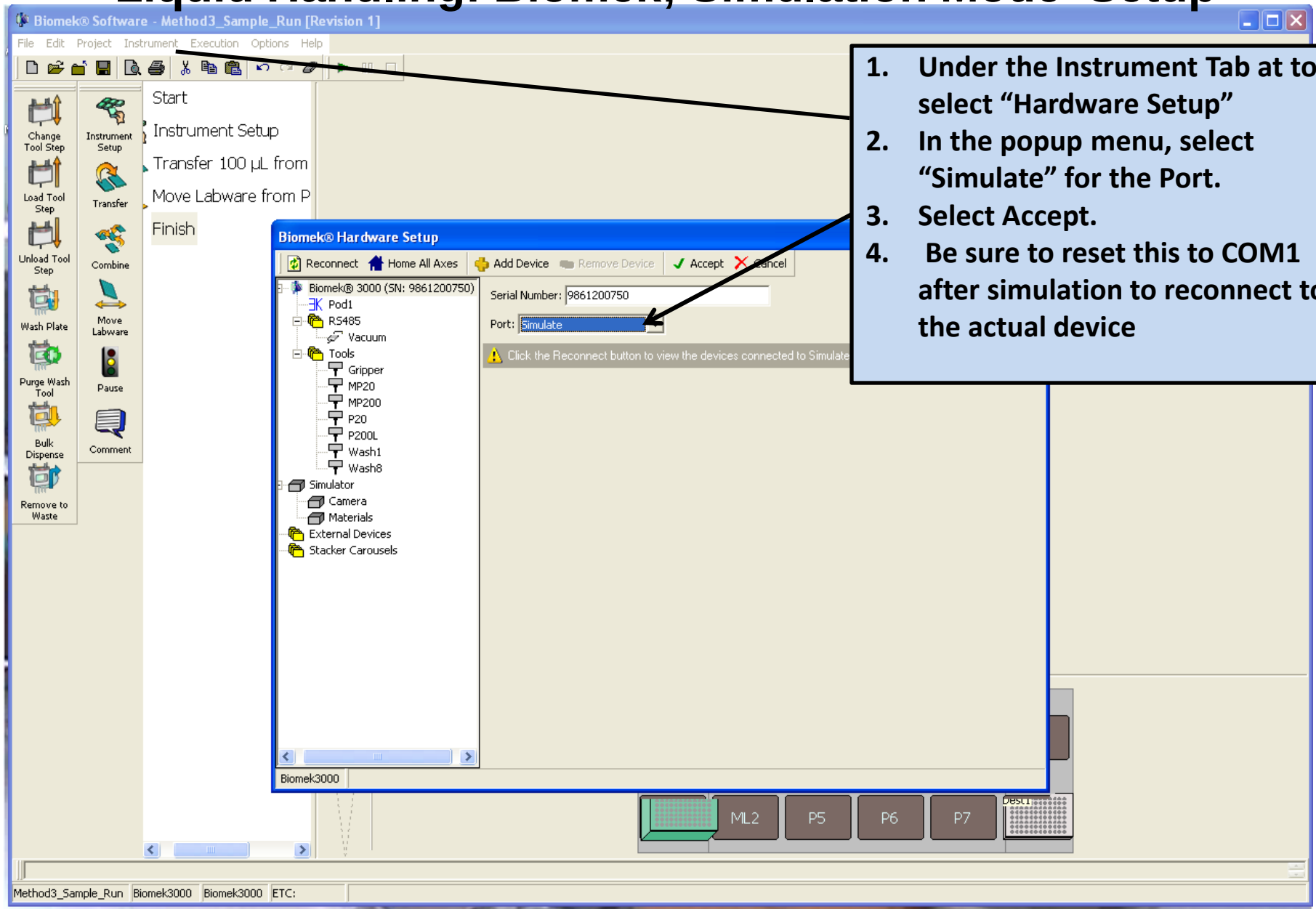


1. **Double-click on Finish in Methods screen and examine Estimated time to complete in status bar at bottom.**
2. **If there are Errors in the method they will be displayed here.**

Liquid Handling: Biomek; Saving this Method

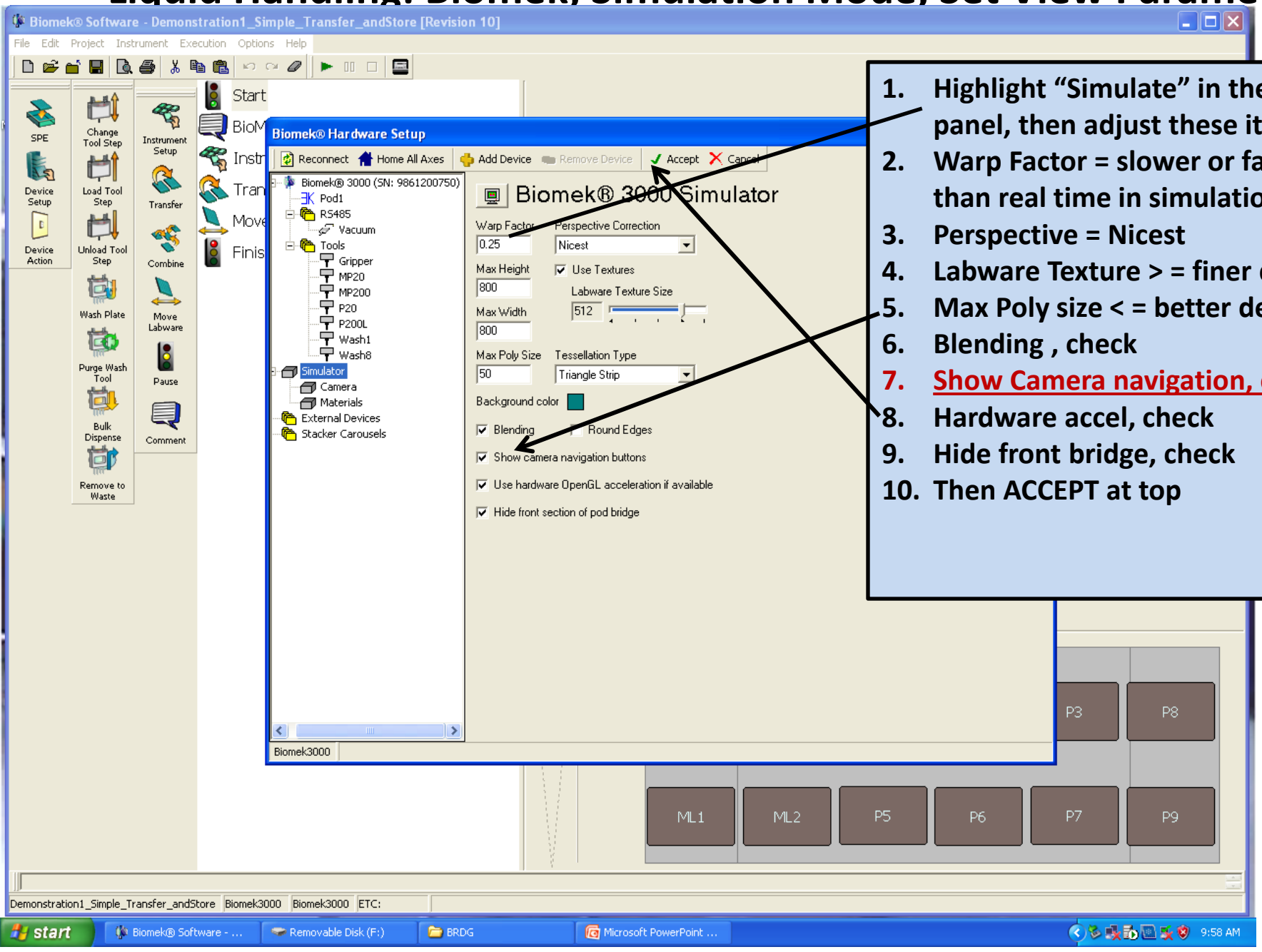


Liquid Handling: Biomek; Simulation Mode Setup



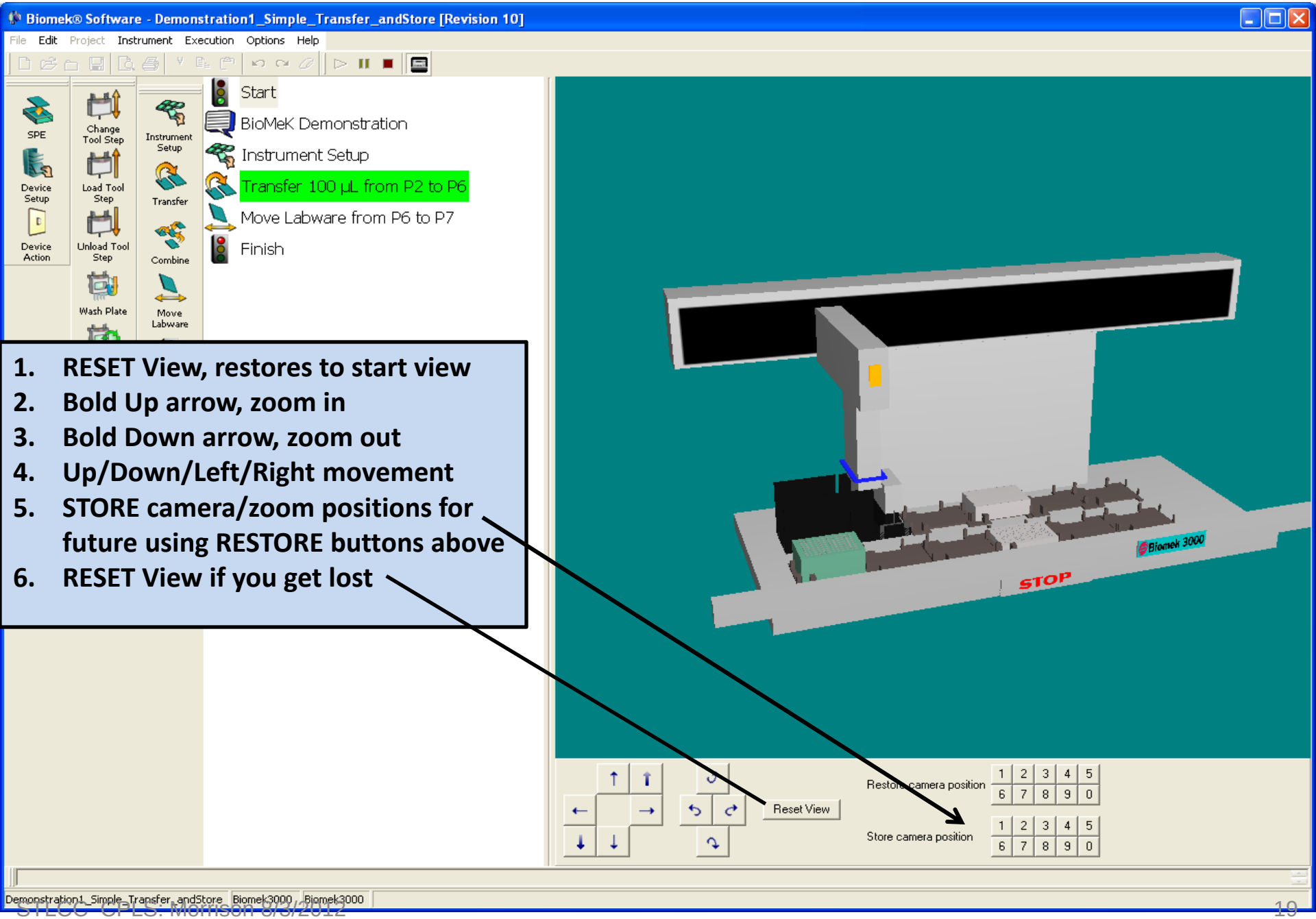
1. Under the Instrument Tab at top, select “Hardware Setup”
2. In the popup menu, select “Simulate” for the Port.
3. Select Accept.
4. Be sure to reset this to COM1 after simulation to reconnect to the actual device

Liquid Handling: Biomek; Simulation Mode, Set View Parameters

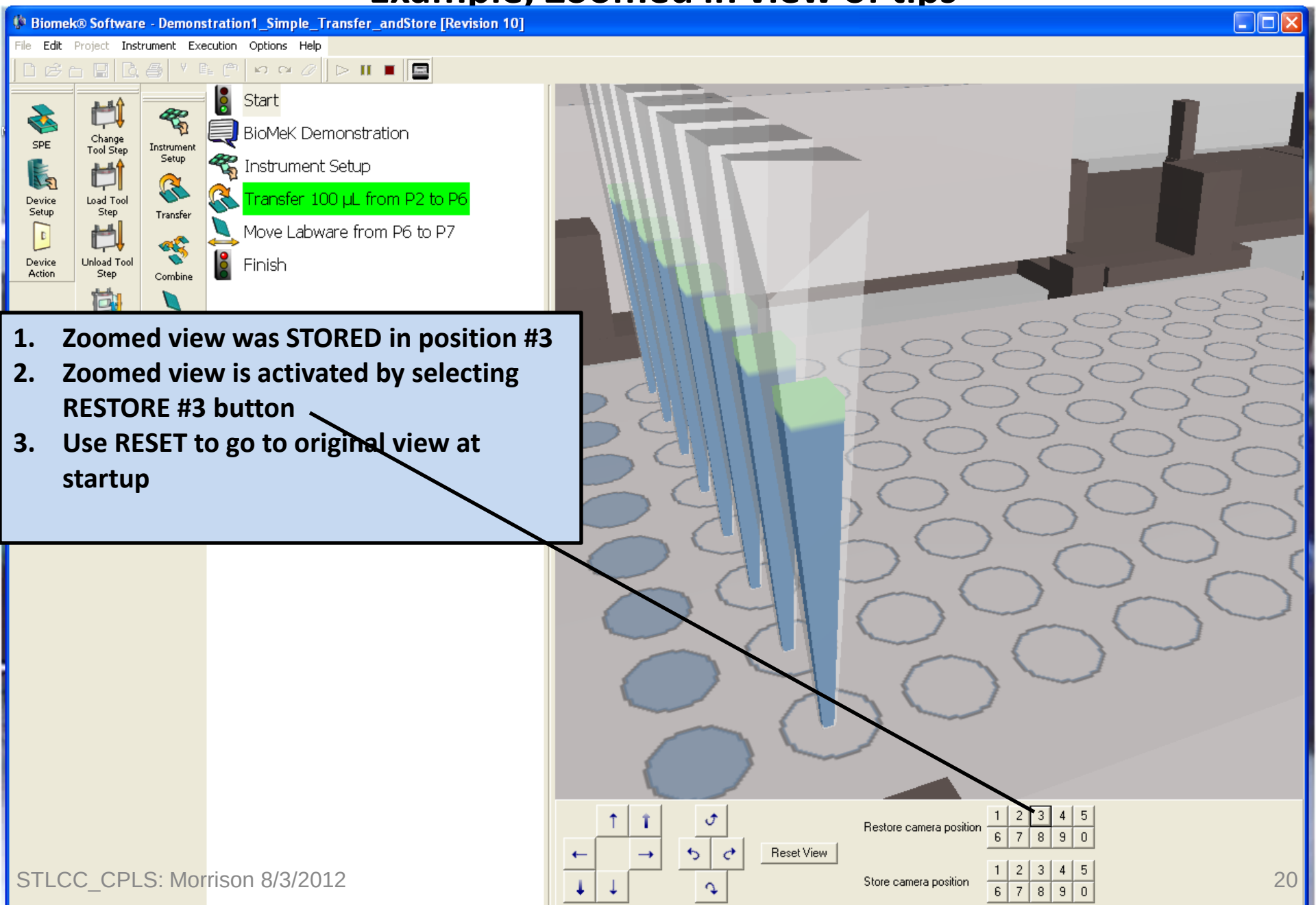


1. Highlight “Simulate” in the left panel, then adjust these items;
2. Warp Factor = slower or faster than real time in simulation
3. Perspective = Nicest
4. Labware Texture > = finer detail
5. Max Poly size < = better detail
6. Blending , check
7. Show Camera navigation, check
8. Hardware accel, check
9. Hide front bridge, check
10. Then ACCEPT at top

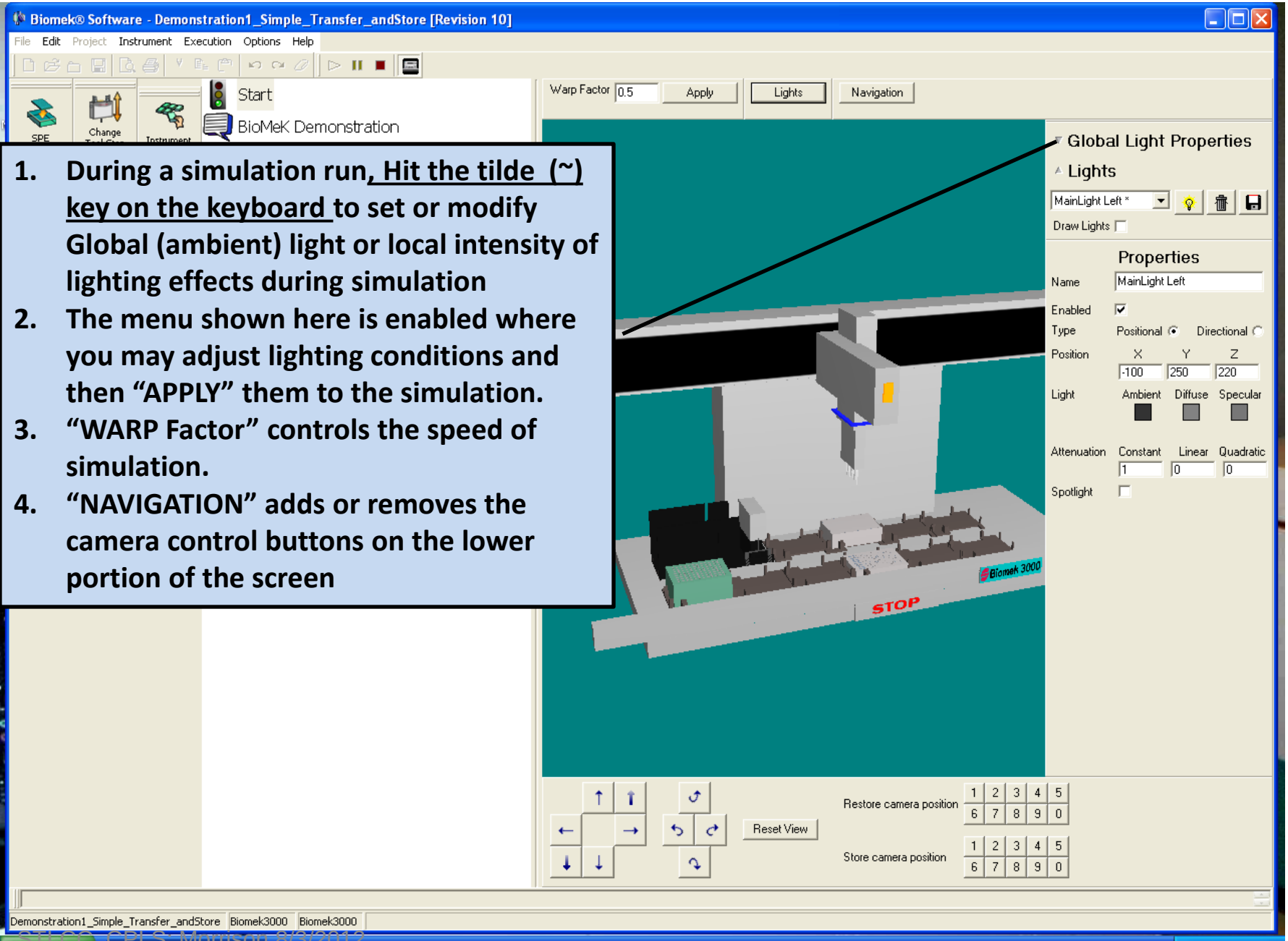
Liquid Handling: Biomek; Simulation, Camera Control Buttons



Liquid Handling: Biomek; Simulation Mode, Restore Button #3 Example, Zoomed in view of tips



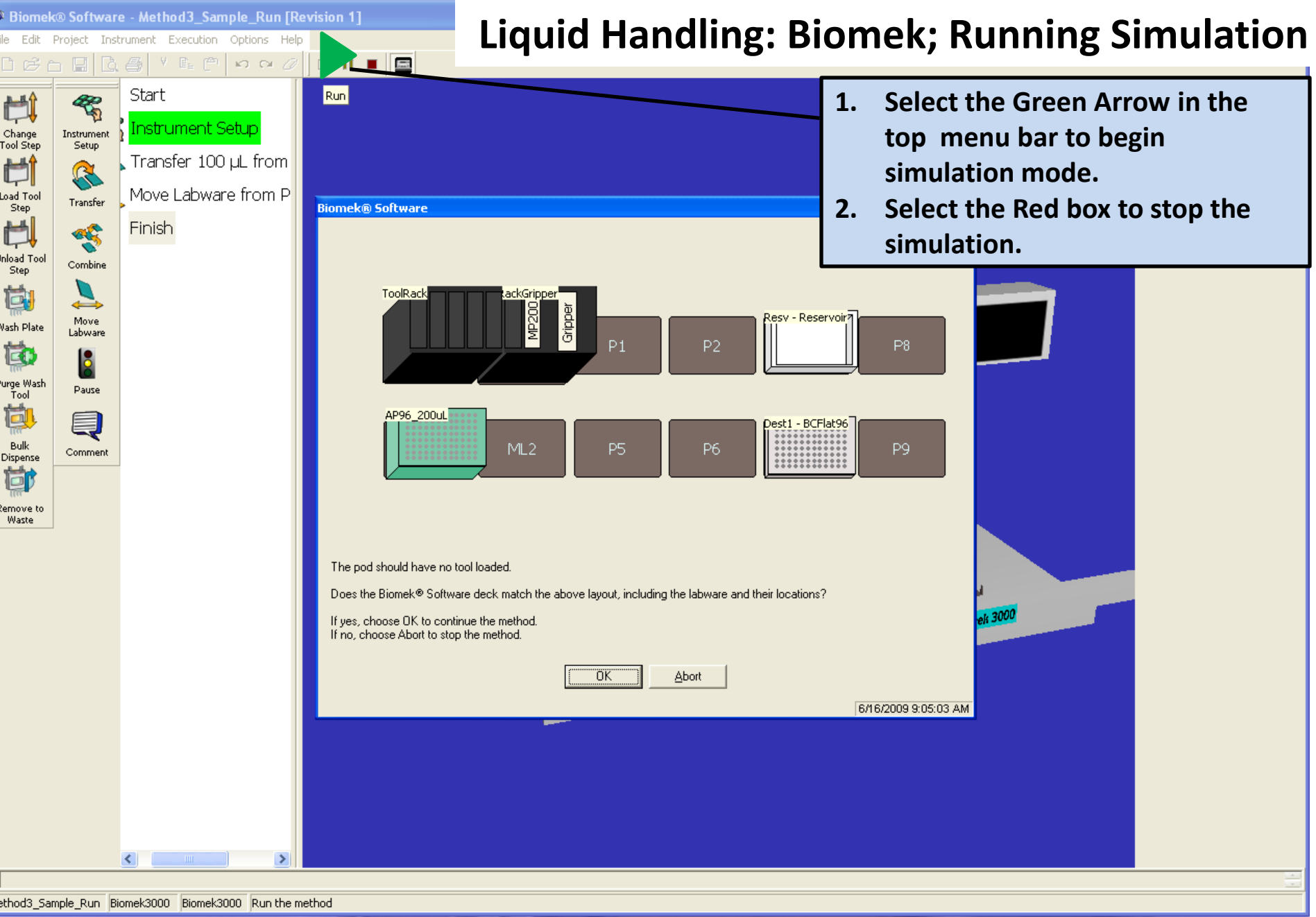
Liquid Handling: Biomek; Simulation, Lighting Control



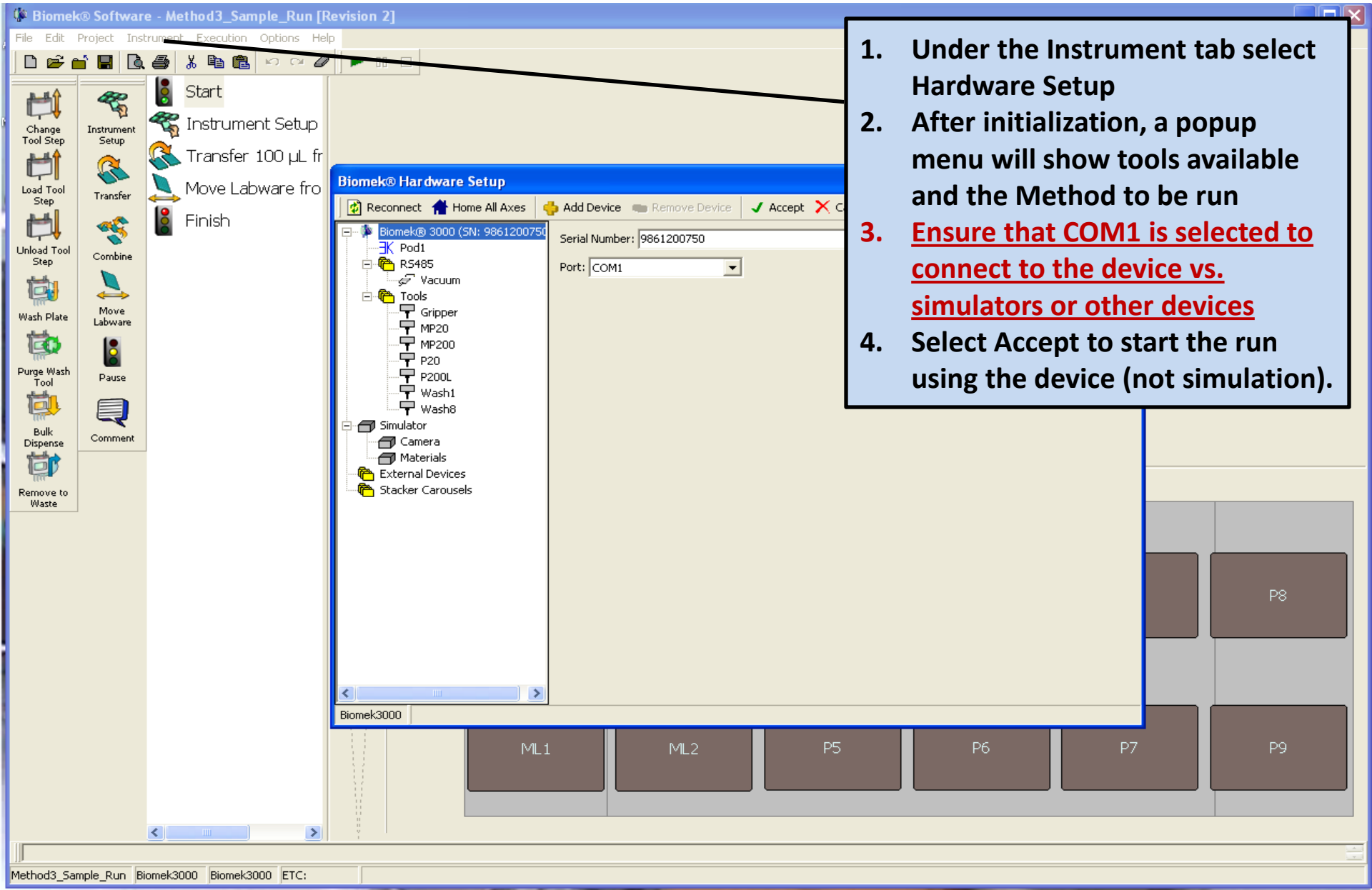
1. During a simulation run, Hit the tilde (~) key on the keyboard to set or modify Global (ambient) light or local intensity of lighting effects during simulation
2. The menu shown here is enabled where you may adjust lighting conditions and then "APPLY" them to the simulation.
3. "WARP Factor" controls the speed of simulation.
4. "NAVIGATION" adds or removes the camera control buttons on the lower portion of the screen

Liquid Handling: Biomek; Running Simulation

1. Select the Green Arrow in the top menu bar to begin simulation mode.
2. Select the Red box to stop the simulation.

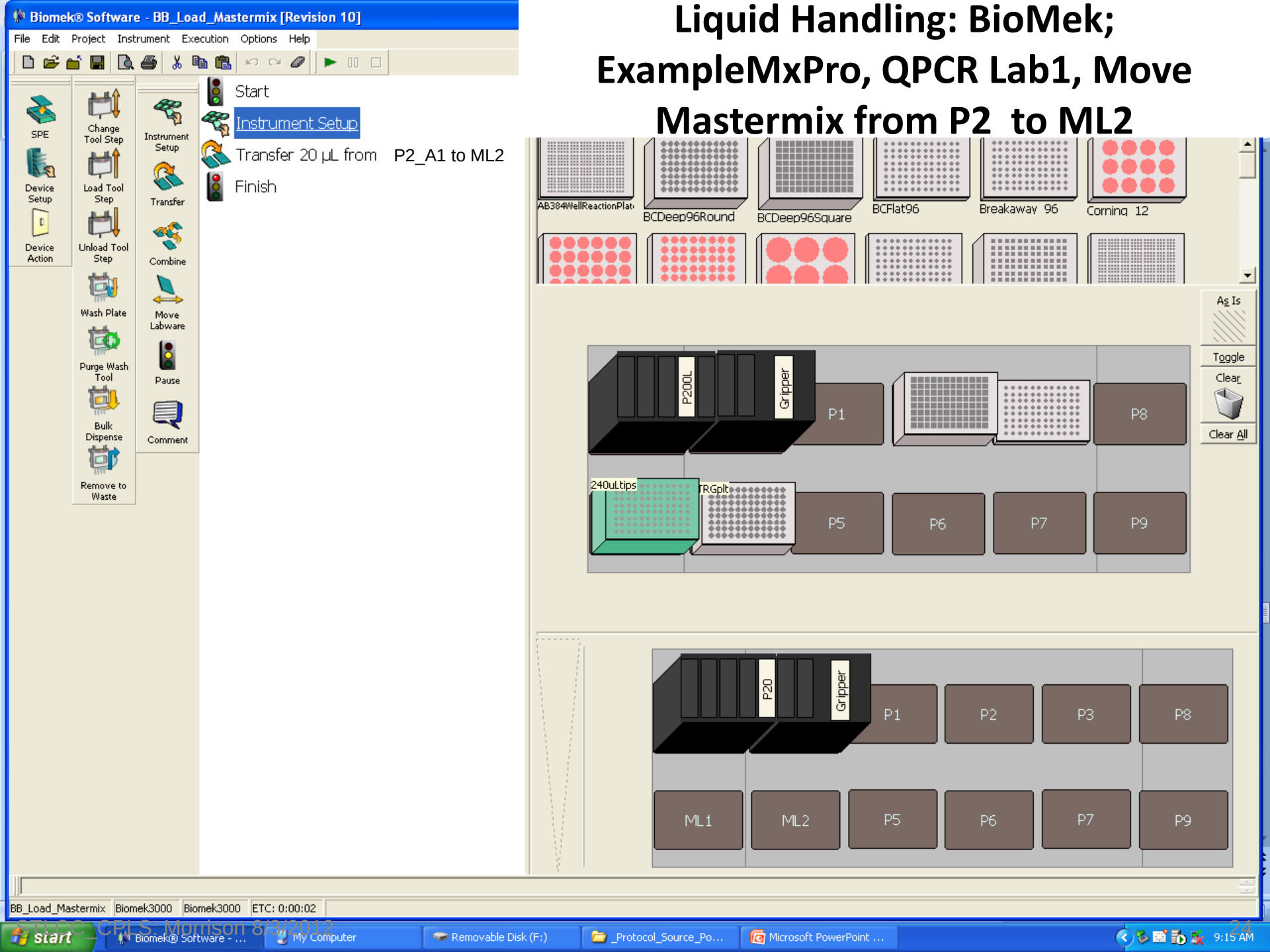


Liquid Handling: Biomek; Running the Method

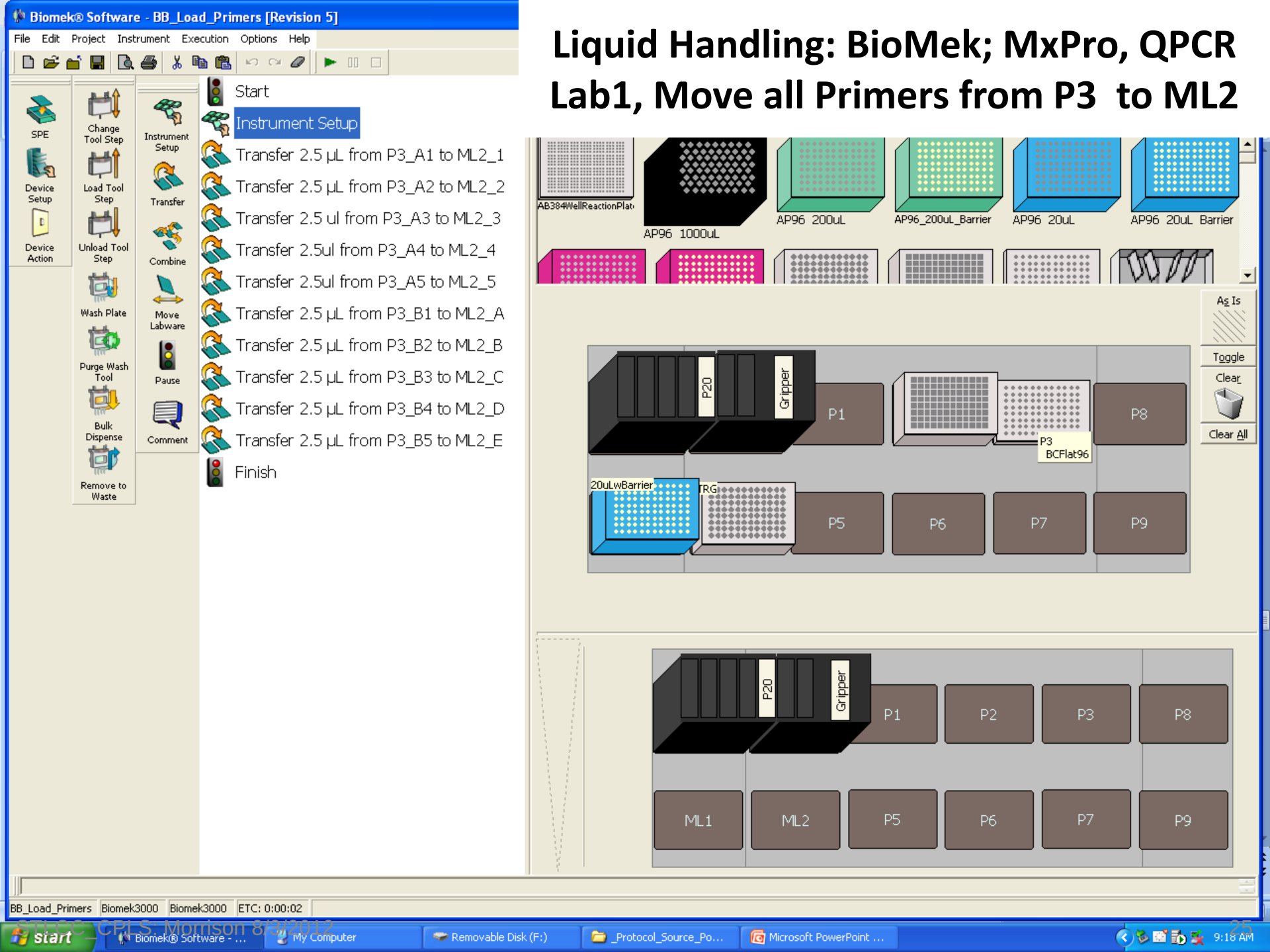


1. Under the Instrument tab select Hardware Setup
2. After initialization, a popup menu will show tools available and the Method to be run
3. **Ensure that COM1 is selected to connect to the device vs. simulators or other devices**
4. Select Accept to start the run using the device (not simulation).

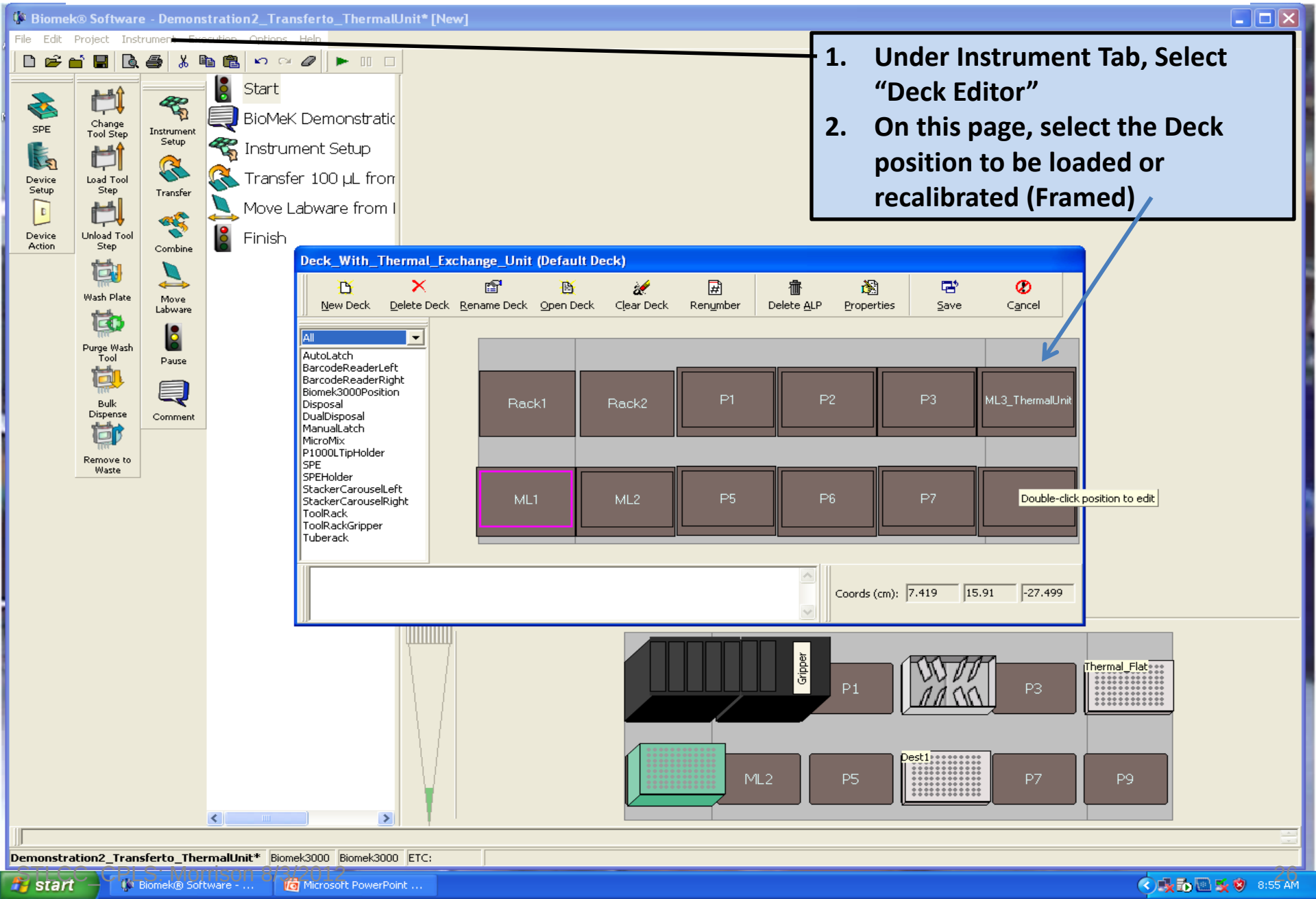
Liquid Handling: BioMek; ExampleMxPro, QPCR Lab1, Move Mastermix from P2 to ML2



Liquid Handling: BioMek; MxPro, QPCR Lab1, Move all Primers from P3 to ML2



Liquid Handling: BioMek; Adding Deck Hardware/Devices



Liquid Handling: BioMek, Maintenance, Calibrating X-Y, Weekly

12.2.1 Calibrating the X- and Y-Axes Positions

The drive cables of the Biomek 3000 workstation stretch and change over time. These changes in the drive cables can cause the movements estimated by Biomek Software to position the pod in a desired location to require slight corrections. As the Biomek 3000 workstation is used, these corrections are needed more frequently, increasing the time required to complete a method.

For this reason, it is recommended to calibrate the X- and Y-axes on a weekly basis. Calibrating the X- and Y-axes on a regular basis limits the frequency that corrections are required.

To perform a position calibration on the X- and Y-axes:

1. In Biomek Software, from the Instrument menu, choose **Hardware Setup**. Hardware Setup appears.
2. In the left pane of Hardware Setup, select **Pod1** to display the Biomek 3000 pod configuration (Figure 12-3).

WARNING: Keep clear of the Biomek 3000 work area when a position calibration is in progress. The bridge and head assembly move back and forth across the deck.

3. Choose **Calibrate X&Y Axes** to calibrate the X- and Y-axes of the Biomek 3000 workstation. An automatic calibration of the X- and Y-axes is performed.

Note: The calibration takes approximately one to two minutes to complete. Wait for the calibration to finish before continuing with any other activities.

Liquid Handling: BioMek; Framing Tool Installation

Manually framing the Biomek 3000 workstation requires the framing tool to be installed on the head assembly. The framing tool is a tool with a single probe used to precisely teach the Biomek Software the locations of all labware positioners on the deck.

To attach the manual framing tool to the head assembly:

1. Push a tip onto the probe on the framing tool (Figure 13-1).

Note: Make sure the tip is not bent or damaged even slightly.

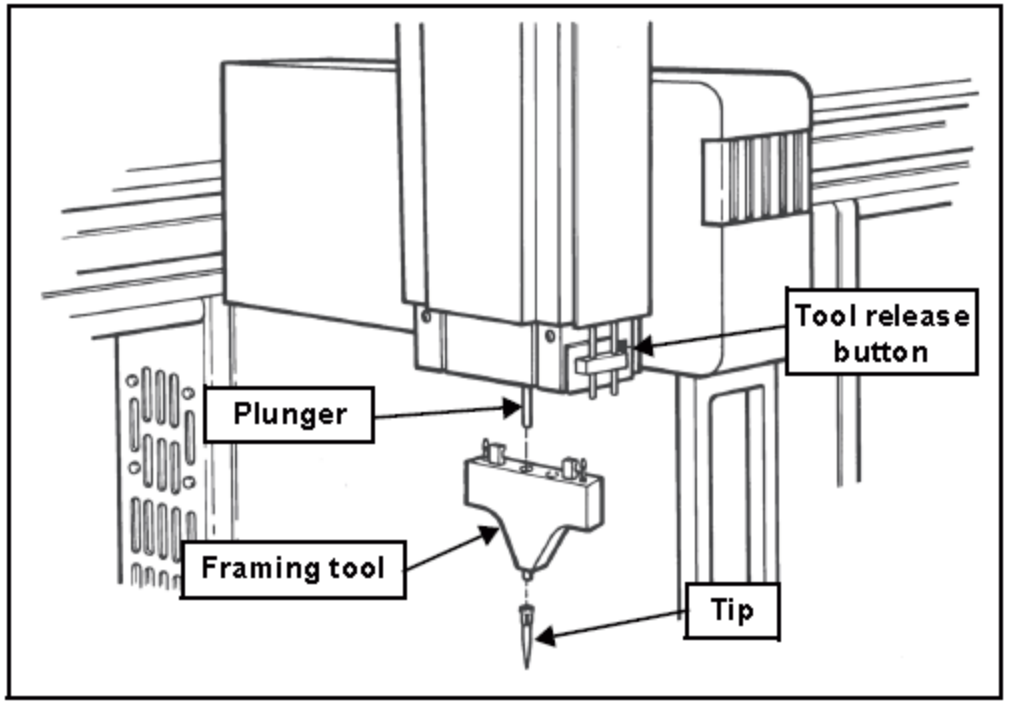


Figure 13-1. Installing the framing tool on the head assembly

2. Press the tool release button on the head assembly and push the framing tool onto the plunger (Figure 13-1).

Note: Make sure that the arrow marked FRONT is pointing towards the front of the Biomek 3000 deck.

3. Release the button when the framing tool is properly seated.

Liquid Handling: Biomek, Framing to Target on each Deck Position

13.2.1 Framing to Position Target

Most labware positioners for the Biomek 3000 have a target position in the back right corner. The target is a small circle, with a larger circle and guide lines to help align the tip to the smaller circle (Figure 13-9). When framing to the target position, the framing tool tip must be aligned to the center of the smaller circle in the middle.

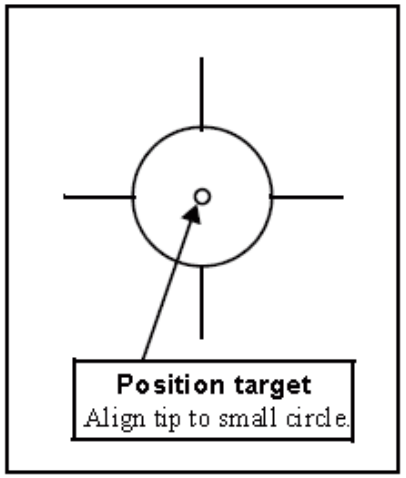


Figure 13-9. Position target on labware positioners

To frame to the target position on the labware positioner:

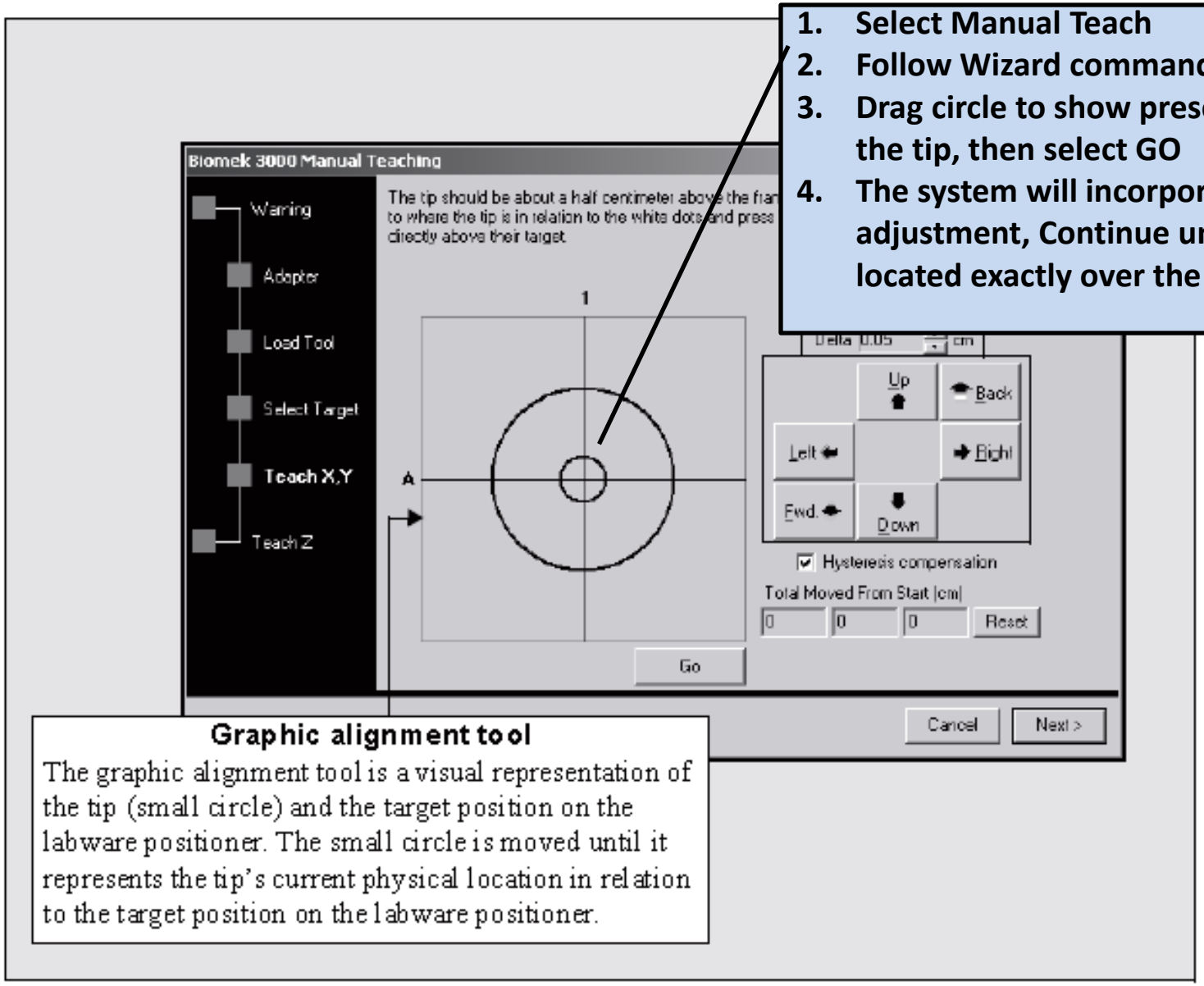
1. Leave Hysteresis compensation at the default setting of **On**. This compensates for any variance along the X-axis from the front of the bridge to the back of the bridge. The Biomek 3000 workstation determines the head assembly's position in the X-axis based on the position of the bridge at the back of the Biomek 3000 workstation (refer to Section 1.2, *System Components*, for more information on the bridge and head assembly).
2. To align the tips in the X- and Y-axes with the target position on top of the labware positioner, lower the framing tool tip in the Z-axis until it is approximately 1 mm above the top of the position.

   Note: Since tip height is set in the next step in the Biomek 3000 Manual Teaching process, it is safe to move the head assembly to any height to make aligning the tips with the target position easier.

3. Visually verify the physical position of the tips in relation to the target position on top of the labware positioner.
4. Adjust the position of the tip to align it with the target position by either:
   - *Using the Graphic Alignment Tool* (Section 13.2.2.1).

Liquid Handling: Biomek, Framing, Manual Teach, X-Y to Target

1. Select Manual Teach
2. Follow Wizard commands
3. Drag circle to show present location of the tip, then select GO
4. The system will incorporate this adjustment, Continue until tip is located exactly over the target center



Graphic alignment tool

The graphic alignment tool is a visual representation of the tip (small circle) and the target position on the labware positioner. The small circle is moved until it represents the tip's current physical location in relation to the target position on the labware positioner.

Figure 13-12. Manual Teaching the X- and Y-axes

13.2.3 Framing the Z-Axis

After the X- and Y-axes are framed, the Z-axis must be framed to ensure aspirate and dispense operations are performed at the desired height. The final screen in Biomek 3000 Manual Teaching is for framing the Z-axis (Figure 13-14).

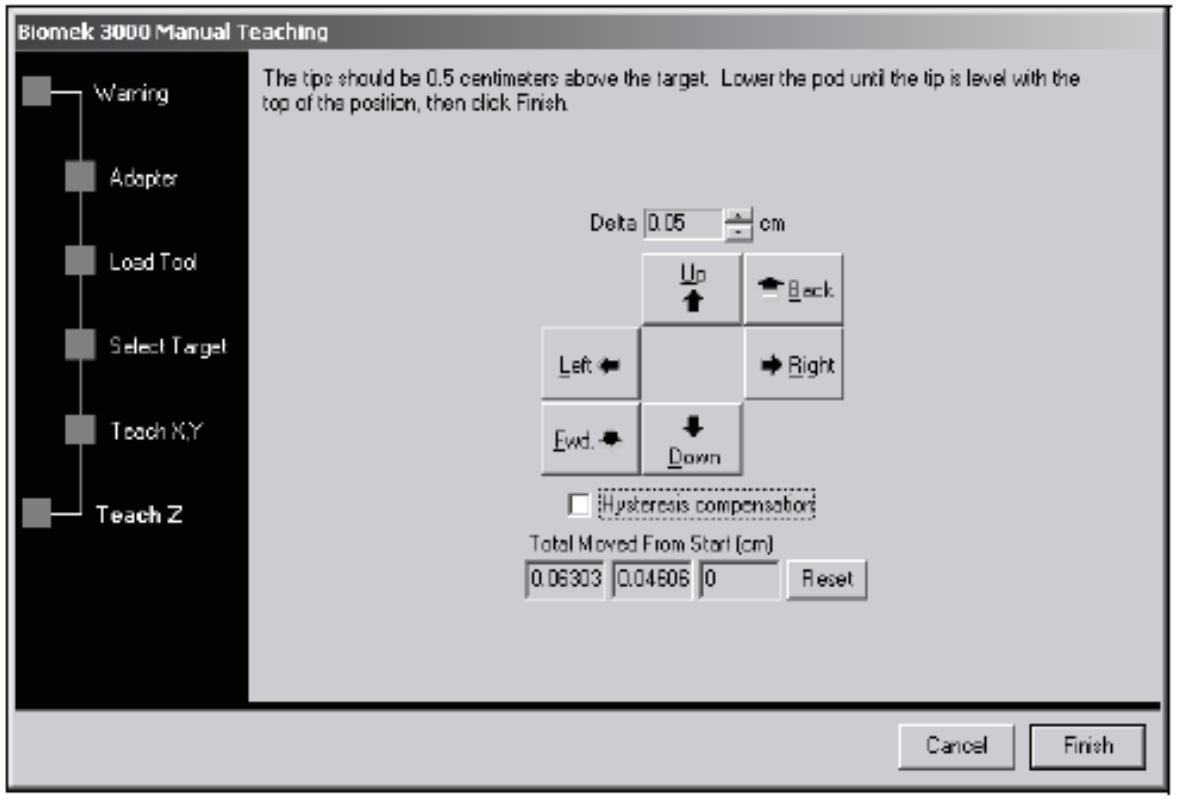


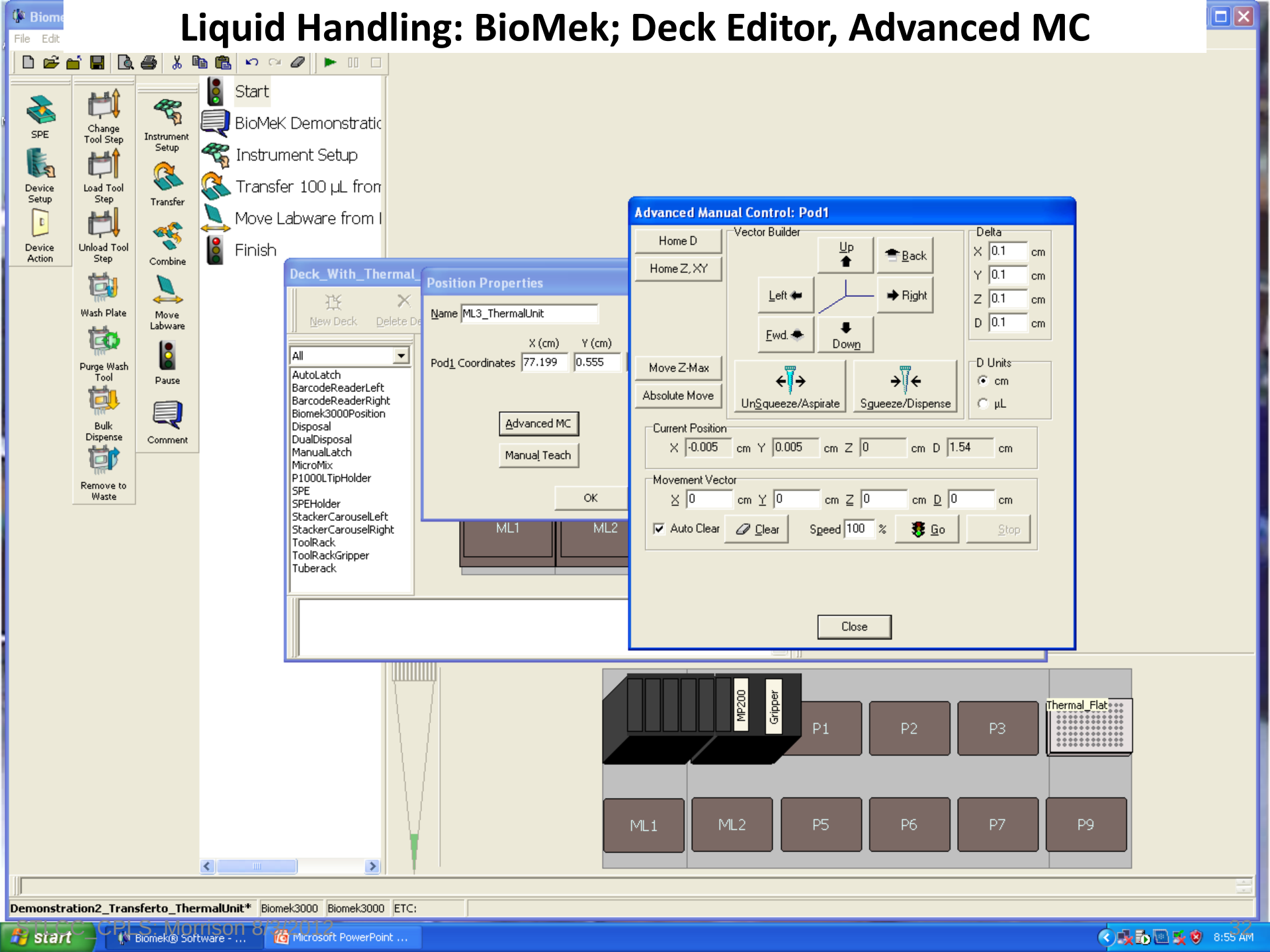
Figure 13-14. Manual Teaching (Teach Z)

1. In **Delta**, select the magnitude of change applied to the framing tool tip each time a directional button is selected (Figure 13-14).

Note: The default Delta value is 0.05 cm. If the tip is a considerable distance above the position, increase the distance traveled by increasing the Delta value (maximum setting is 1.0 cm). If the tip is almost to the desired location, reduce the Delta value (minimum setting is 0.005 cm).

Liquid Handling; Biomek, Framing the Z-Axis

Liquid Handling: BioMek; Deck Editor, Advanced MC



Liquid Handling: BioMek-Manuals

Link to Beckman BioMek3000 User Manual – pdf (15MB)

Link to Beckman BioMek3000 Quick Start Guide – pdf (2MB)

Link to Beckman BioMek3000 Software Manual – pdf (23MB)

Liquid Handling: BioMek; Tip Combatability and Volume Ranges on Tools Section 2.3 in Users Manual

Tool / Tip Type	P20	P20_Barrier	P200	P200_Barrier	P1000
P20 Single-Tip Tool	1–20 µL	1–20 µL	1–20 µL	1–20 µL	--
P200L Single-Tip Tool	1–20 µL	1–20 µL	5–200 µL	5–125 µL	--
P1000L Single-Tip Tool	--	--	--	--	50–1000 µL
MP20 Eight-Tip Tool	1–20 µL	1–20 µL	5–20 µL	5–20 µL	--
MP200 Eight-Tip Tool	1–20 µL	1–20 µL	5–200 µL	5–125 µL	--

Liquid Handling: BioMek; Labware and Tool/Tip Compatability

Tool	Tip Type	Labware Type							
		Flat 96	Deep 96	Flat 384	Deep 384	10 mm Tubes	12 mm Tubes	13 mm Tubes	Modular Reservoir
P20	P20	Y	L[a]	Y	L[a]	L[b]	L[b]	L[a]	Y
	P250	Y	Y	Y	L[b]	Y	Y	L[b]	Y
P200L	P20	Y	L[a]	Y	L[a]	L[b]	L[b]	L[a]	Y
	P250	Y	Y	Y	L[b]	Y	Y	L[b]	Y
P1000L	P1000	Y	Y	N	N	Y	Y	Y	Y
MP20	P20	Y	L[a]	Y	L[a]	N[c]	N[c]	N[c]	L[a]
	P250	Y	Y	Y	L[b]	N[c]	N[c]	N[c]	Y
MP200	P20	Y	L[a]	Y	L[a]	N[c]	N[c]	N[c]	L[a]
	P250	Y	Y	Y	L[b]	N[c]	N[c]	N[c]	Y

Y = Full Access — can reach the bottom of wells.
N = No Access — cannot enter wells.
L = Limited Access due to height and/or width restrictions (see Caution above)

a. Tip is too short to access bottom of well or tube.
b. The maximum diameter of the tip is too wide for the tip to fully access the wells or tubes.
c. Probe spacing on the multi-channel pipette tools prevents access into test tubes.

Liquid Handling: Shaker Integration Kit

DPC Shaker Integration Kit for Biomek 2000/3000 , Part No 380561
(380713 CD, 380535 Mplatex2, 380534 standoff x 4, 380535 cable)

Includes mounting brackets, support legs for Biomek 2000/3000, shaker top plate, two shaker labware holders, communications cable, instructions and installation.

Refer to Appendix C of the Users Manual for details on attachment, assembly, and operation.

The DPC Micromix 5 Shaker is part No 380560 , a separate component, but requires the integration kit (380561). This device is ordered through and shipped directly by Beckman Coulter. DPC (Diagnostics Products Corporation has been acquired by Siemens) which apparently no longer sells the Shaker.

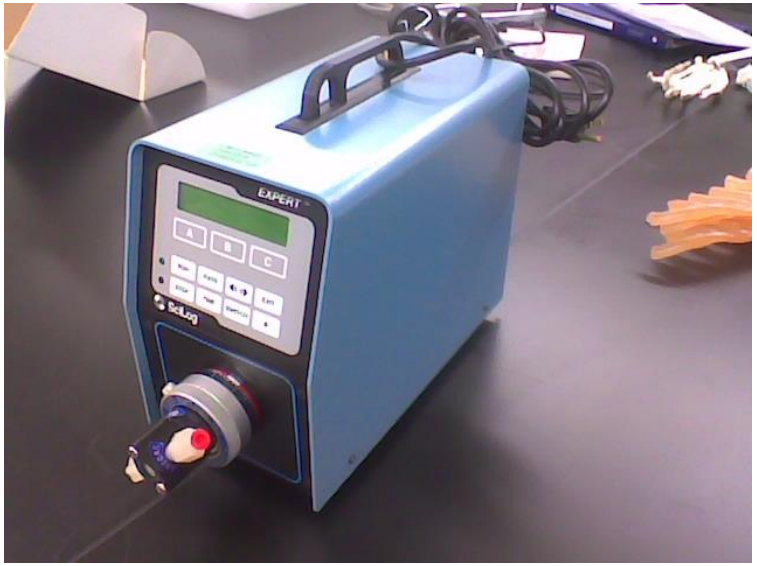
Liquid Handling: BioMek3K, Thermal Exchange Unit Required and Pump

Biomek® Thermal Exchange Unit and Reservoir Holder Kit

The Biomek 2000/Biomek 3000 Thermal Exchange Unit (TEU) accessory heats or cools a reservoir, microplate or other standard labware on the Biomek 3000 work surface. Labware placed on the deck.

TEU can be maintained at temperatures from 0° to 50°C. The TEU comes with a metal Thermal Reservoir Holder for standard Biomek reservoirs.

Includes Thermal Exchange Unit, Thermal Reservoir Holder, Tubing and Manual.
Requires BioWorks 2.1 or later.



SciLog Peristaltic Pump (shown)

The Circulating Reservoir provides a continuous supply of buffers, solvents or reagents on the Biomek worksurface. A four-well Teflon* reservoir is placed in one position on the Biomek worksurface. A peristaltic pump circulates liquid between two wells of the reservoir and supply bottles.

Liquid Handling: Plate Washer, Bio-Rad Bio-Plex Pro II

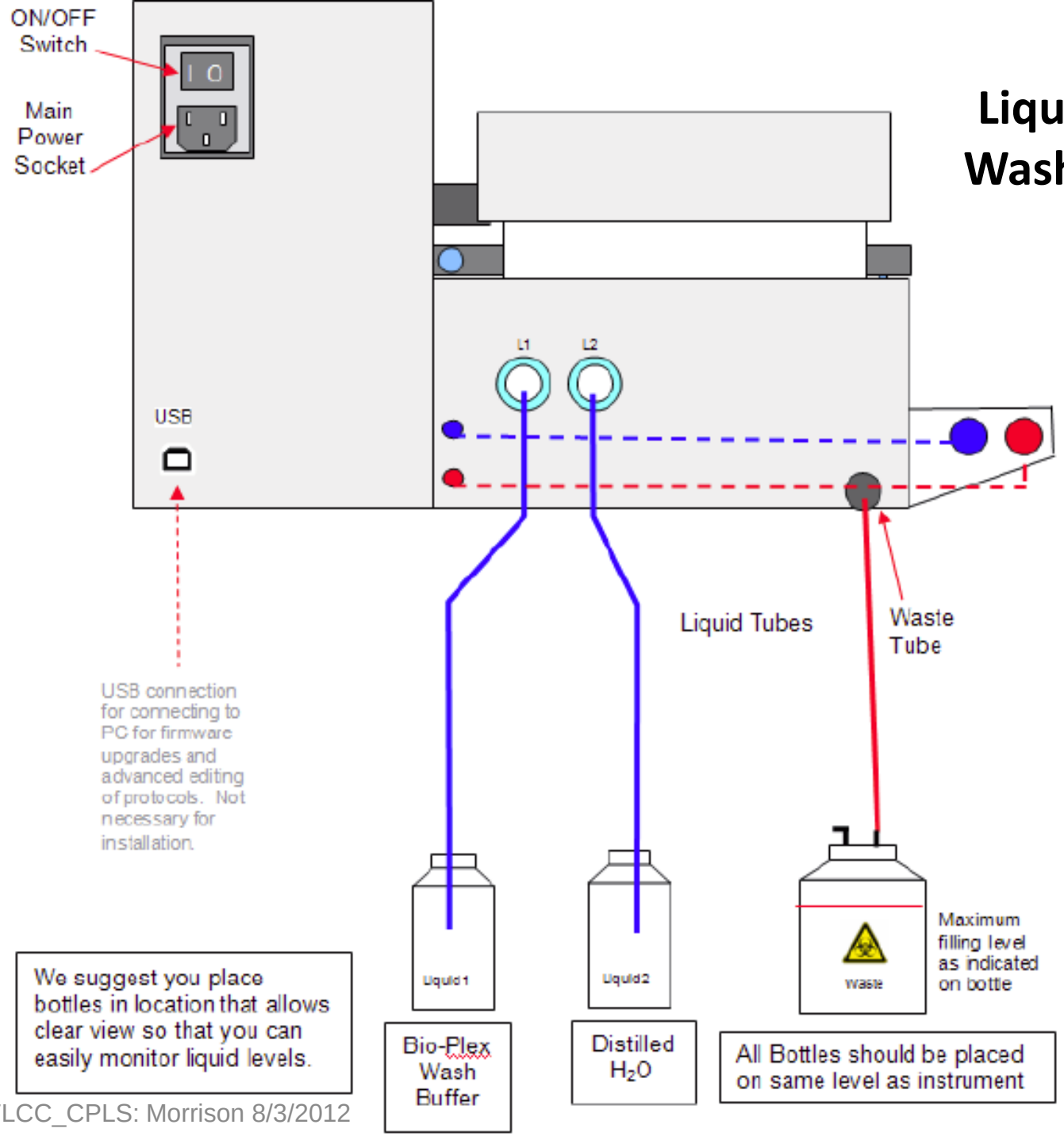
Input voltage range 100–240 V, 1.8 A, 47 – 63 Hz
Physical dimensions 44 x 61 x 8 cm (W x D x H)
Weight 14.4 kg (32 lb)
Communications interface RS – 232
Plate capacity One 96 –well microtiter plate "≤0.75"

•Bio-Plex Pro II wash station — includes both a magnetic carrier and an interchangeable vacuum manifold for hands-free wash steps on any xMAP assay (magnetic and nonmagnetic bead-based assays)

Both options include preset wash programs that have been optimized for Bio-Plex assays. Using Bio-Plex Pro wash stations in an assay workflow can reduce manual intervention and help decrease variability between independent experiments.
Eliminates manual wash steps from Bio-Plex assays, making Bio-Plex assays as easy as ELISAs. The wash stations are specifically designed to perform Bio-Plex assay wash steps, but are compatible with any standard xMAP assay.

Hot link to Bio-Rad Bio-Plex Pro II User Manual…. Pdf (3mb)

Liquid Handling: Plate Washer, Bio-Plex Pro II, Configuration

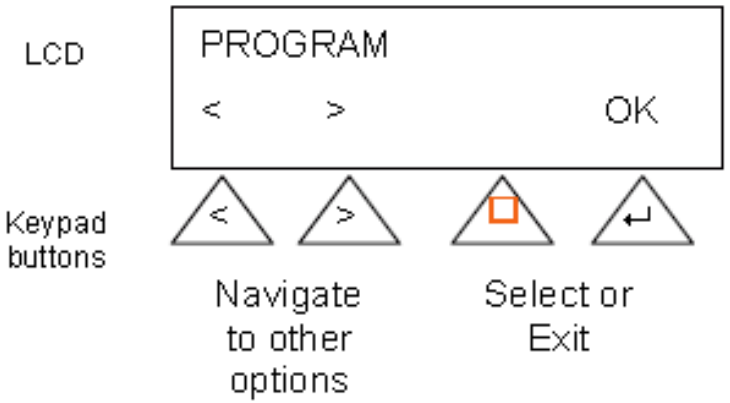

ON/OFF Switch
Main Power Socket
USB
L1
L2
Liquid Tubes
Waste Tube
USB connection for connecting to PC for firmware upgrades and advanced editing of protocols. Not necessary for installation.
Liquid1
Liquid2
Waste
Maximum filling level as indicated on bottle
We suggest you place bottles in location that allows clear view so that you can easily monitor liquid levels.
Bio-Plex Wash Buffer
Distilled H_2O
All Bottles should be placed on same level as instrument

Liquid Handling: Bio-Plex Pro II, Primary and Secondary Menus

Primary Menu Options	Functions	Secondary Menu Options
Programs	Wash programs used to process assays	Start, Define/Edit, Show, Clear
Settings	Rarely accessed advanced features	Edit Plates, Options, Bubble Sensor
Procedures	Preparation or maintenance processes	Prime, Rinse, Empty Prime Tray, Vacuum Filtration

Secondary Menus; Programs example

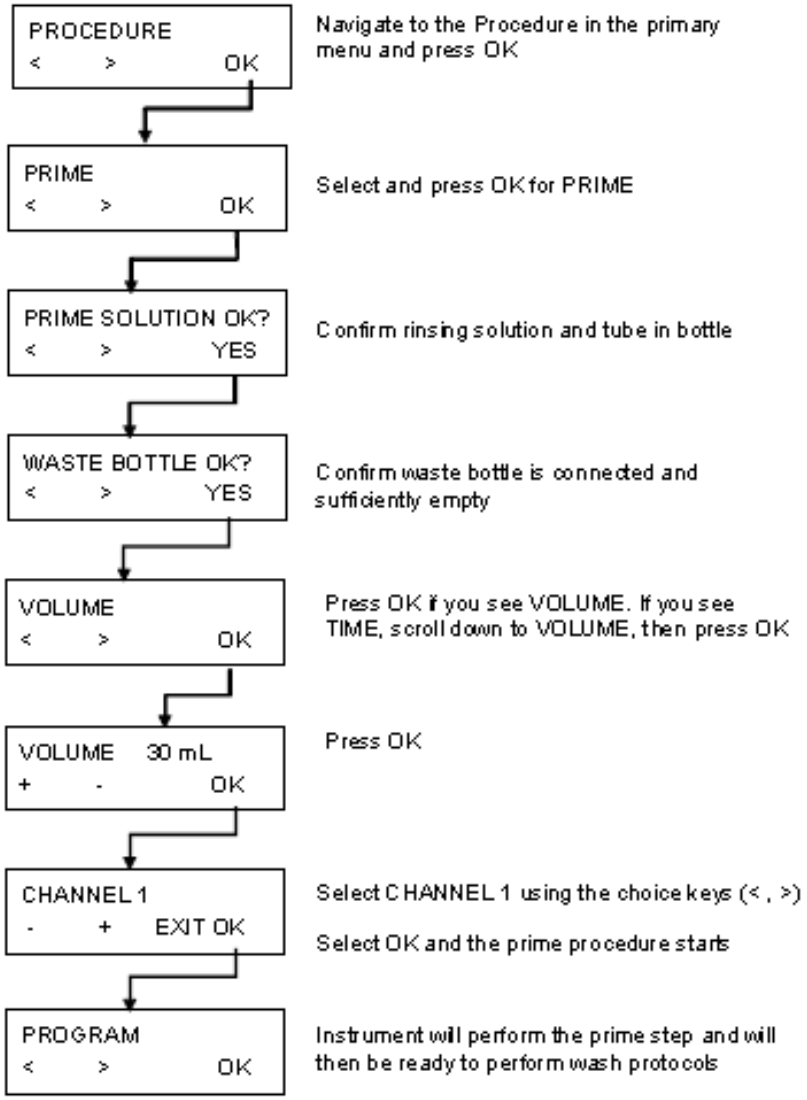
If you choose OK when any of the primary menu options (Programs, Settings, and Procedures) are visible on the LED, you will navigate to the secondary menus. You may Select a choice from the secondary menu, or press Exit to return to the primary menu.



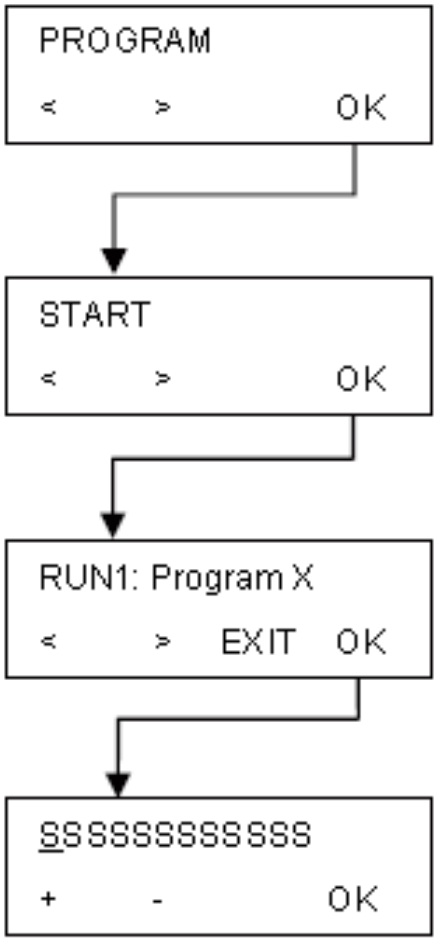
If you get confused about where you are, press Exit, until you no longer see Exit as an option.

Liquid Handling: Plate Washer, Bio-Plex Pro II Priming

You should prime both Channel 1 and 2 after installation and if the instrument has not been used for an extended period of time. Priming is performed to fill the liquid system of the instrument and to remove all air from the tubes. A priming step must also be performed when switching to a different wash buffer.


PROCEDURE
< > OK
Navigate to the Procedure in the primary menu and press OK
PRIME
< > OK
Select and press OK for PRIME
PRIME SOLUTION OK?
< > YES
Confirm rinsing solution and tube in bottle
WASTE BOTTLE OK?
< > YES
Confirm waste bottle is connected and sufficiently empty
VOLUME
< > OK
Press OK if you see VOLUME. If you see TIME, scroll down to VOLUME, then press OK
VOLUME 30 mL
+ - OK
Press OK
CHANNEL 1
- + EXIT OK
Select CHANNEL 1 using the choice keys (< , >)
Select OK and the prime procedure starts
PROGRAM
< > OK
Instrument will perform the prime step and will then be ready to perform wash protocols
Repeat for channel 2

Liquid Handling: Bio-Plex Pro II, Selecting Wash Program



Navigate to PROGRAM in the primary menu and press OK.

Use the keys under the < and > symbols to access the Program menu if it is not already displayed

Press OK

Select the required program and press OK to continue

Choose OK.

Rows are represented by S (selected) or N (not selected). All rows should be selected.

It is highly recommended that all wells are selected and the entire plate is washed.

Liquid Handling: Bio-Plex Pro II, Which Wash Program?

Which Wash Program Do I Use?

In this instance.	Use Wash Station Program
First wash, magnetic assay	MAG x2
All other washes, magnetic assay	MAG x3
Prewash, vacuum assay	PREVAC
First wash, vacuum assay	VAC x2
All other washes, vacuum assay	VAC x3
Third party kits which require 200 µl washes (Vacuum only)	V 200 x2 or V 200 x3

For all protocols, it is highly recommended that you wash the entire plate. See page 18 for details.

Liquid Handling: BioPlex, Quick Guide to Wash Programs

Manual Steps	Wash Station Protocols
<u>Vacuum Program Only</u> Dispense 125uL Assay Buffer Perform Prewet Program	PREVAC
Dispense 50uL Bead suspension Wash 2 cycles	MAG x2 or VAC x2
Dispense 50uL standars and solutions *Incubate for 30-120 minutes. (Refer to assay manual) Wash 3 cycles	MAG x3 or VAC x3
Dispense 25uL detection antibody *Inubate for 30 minute Wash 3 cycles	
Dispense 50uL SA-PE *Incubate for 10 minutes Wash 3 cycles	
Dispense 125uL Assay Buffer Place on shaker and shake for 1 minute Read on Bio-Plex Reader	

*Incubating reaction plates should be
- At room temperature
- Covered with aluminum foil, or otherwise protected from light
- Shaken at 850 ± 100 rpm for the first 20 seconds, and at 300 rpm for the remainder of the incubation

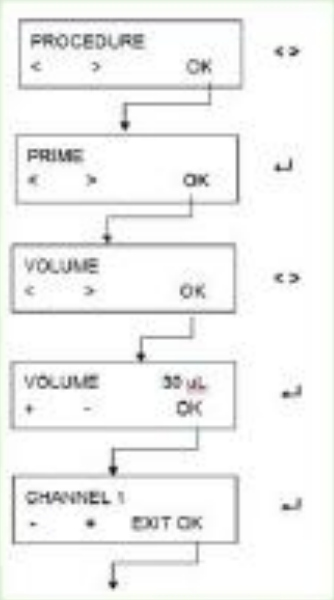
Liquid Handling: BioPlex Pro, Using Wash Station for Daily Operations

1. Prime Procedure

CAUTION: Do not run any programs until you have primed channel 1.

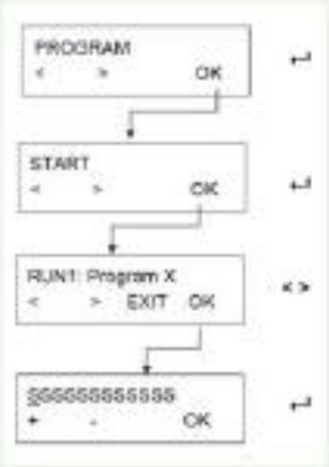
Prime Channel 1 if

1. Your last action was a rinse with Channel 2
2. The system has been idle for more than an hour
3. You are changing buffer in bottle 1



Repeat for Channel 2

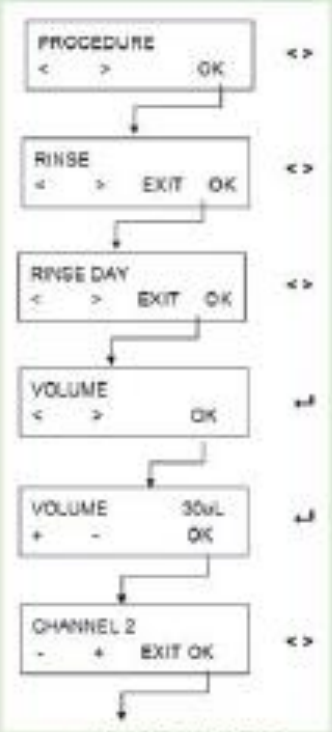
2. Wash Programs



3. Rinse Procedures

Use RINSE DAY if you will use the instrument later that day.

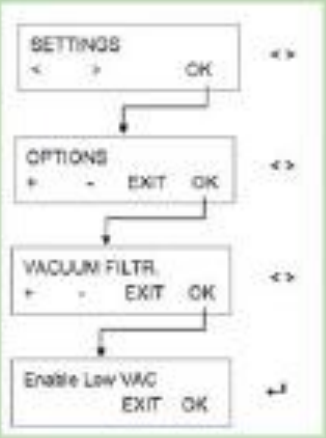
Use RINSE NIGHT at the end of the day



or RINSE NIGHT

Bio-Plex Pro II Only

Enable/Disable vacuum carrier



Clean your vacuum plate carrier daily

Key

< > Select option shown
↵ Use default option

Liquid Handling: Bio-Plex Pro, Short Term Maintenance

Daily

- Prime the liquid system if necessary
- Perform Rinse Day with wash buffer, if the instrument is left to stand for a short time (up to 2 hours). Use distilled water if you anticipate longer intervals between uses
- Perform Rinse Night with distilled water at the end of a day of use
- If necessary (due to particles, soiling, etc.), prime the instrument several times with distilled water
- If the instrument will be left to stand for a longer period of time (more than one week), prime with distilled water and then prime without liquid

Weekly

- Perform Rinse Night with distilled water and soak the manifold overnight (leave instrument on)
- Prime the instrument without liquid to empty the entire liquid system. (Reconnect the lines and prime with distilled water unless not using the instrument for an extended period)
- Check the filter(s) in the liquid bottle(s) for particles and rinse liquid filter(s) with distilled water
- Clean the plate carrier guide bar with 70% ethanol

Liquid Handling: Bio-Plex, Long Term Maintenance

Every Six Months

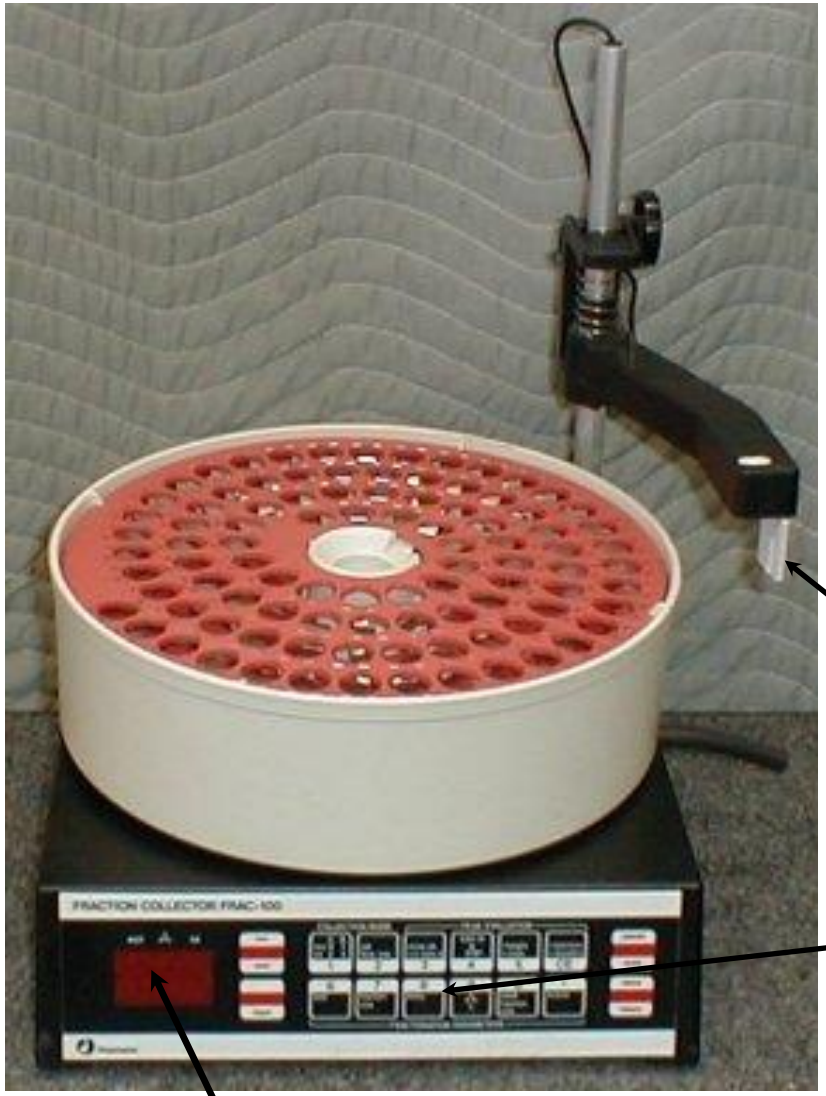
- Clean the plate carrier guide bar with 70% ethanol
- Remove the magnetic plate carrier and check the spring-loaded centering mechanism at the right rear of the carrier. If the mechanism does not move freely, rinse thoroughly with 70% ethanol
- Clean aspiration and dispensing needles. Two kinds of cleaning needles are supplied with the instrument. Clean the manifold aspirating needles with the smaller of the two. Clean the dispensing needles with the thicker cleaning needles. Carefully raise the manifold arm and clean each instrument needle by inserting the cleaning needle into the instrument needle and gently agitating, to remove any possible debris. Then rinse with water
- If you choose to remove the manifold for cleaning refer to page 66 for instructions

As Needed

To replace the plate carrier seal:

1. If the plate carrier seal becomes visibly damaged, remove the defective seal and clean the plate carrier frame with ethanol, making sure no adhesive is left
2. Peel back the protective paper from the new seal and attach to the plate carrier frame be careful not to block the ventilation hole

Fraction Collection; FRAC-100,200



- Peak detection and location capability, programmable collection modes.
- Communication with external components, tube and level sensors.
- Chromatogram evaluation function real-time integration of peak height, area
- 95 places rack for 10-18mm tubes
- Time mode 0.01-9.999 min, volume mode 0.01-9.999 ml
- Pharmacia part # 19-6000-01

Delivery Arm

Setup, Collection Mode, Control Keys

Alphanumeric Display

Fraction Collector: Definition

- Fraction Collector A fraction detector is a device that allows regular or specified samples to be taken from a column eluate and stored in a retrievable form. The storage vessels are usually small sample tubes or vials that are oriented in a rotating disk or in a moving belt, there movement usually being controlled by a microprocessor.
- On receiving a signal from the microprocessor, the next vial is placed under the column outlet and the eluate collected until receiving another signal from the computer. Once the properties of the chromatogram that describes the separation has been ascertained, then the collection program can be defined.
- The fractions can be collected on a basis of time either at regular intervals or a specific times to collect specific peaks. Alternatively the fractions can be collected by monitoring the detector output and when a peak starts to elute the fraction collector is activated and the peak collected in a specific vial. When the peak returns to base line the column eluate is then directed to waste until the next peak starts eluting.
- Fraction collectors are in common use with most liquid chromatographs. They are used to collect samples for further purification, subsequent exmination by spectroscopic techniques or for biological or organoleptic testing.